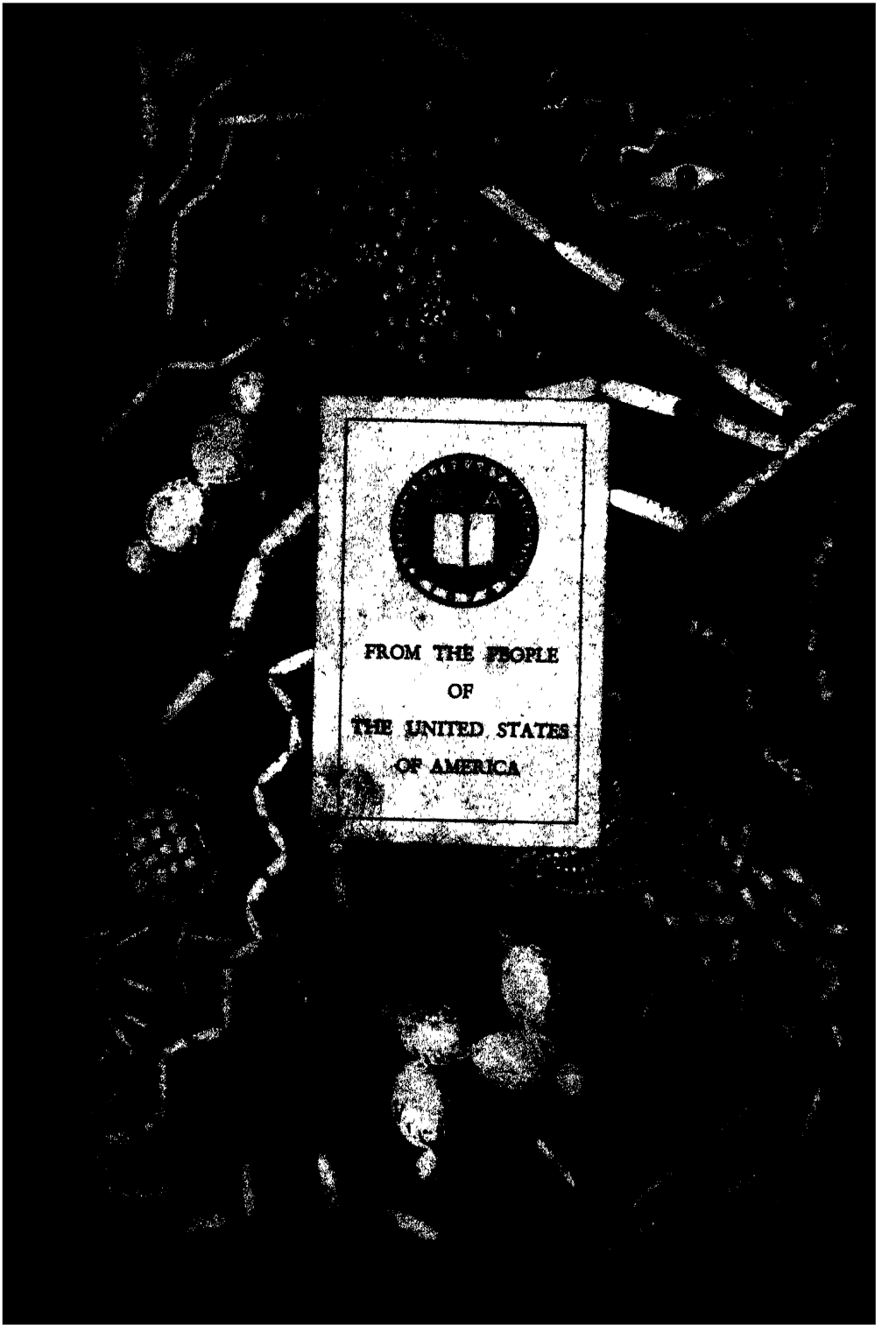
FROM THE PEOPLE
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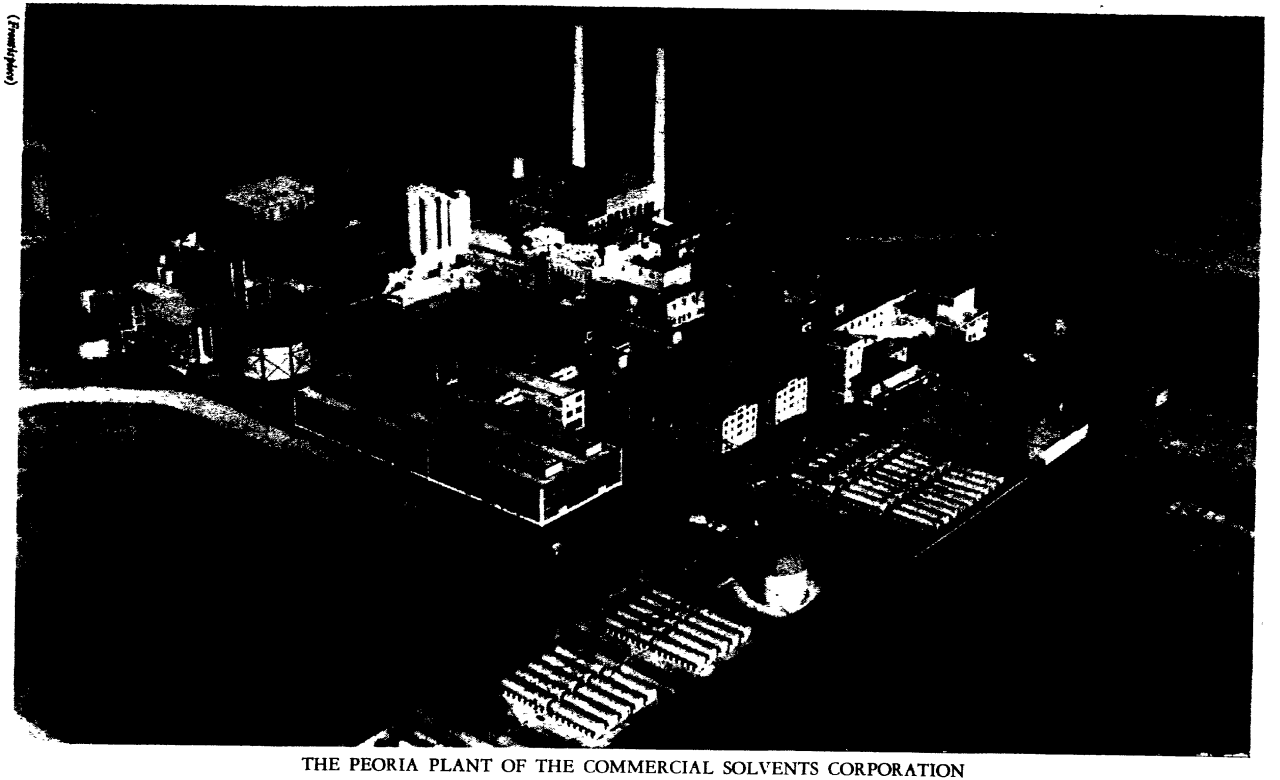
THE UNITED STATES
OF AMERICA

MICROBES OF MERIT



THE PEORIA PLANT OF THE COMMERCIAL SOLVENTS CORPORATION

This factory was built to make certain bacteria ferment grain or molasses to valuable products for the chemical industries. The horizontal tanks in the foreground contain the liquid products while the gas produced by the bacteria goes to the gas tanks at the left. The bacteria in this factory can ferment the grains from 600 acres of land in one day (see Chapter Nineteen).

MICROBES OF MERIT

by OTTO RAHN
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FOREWORD

Microbes are an essential part of our civilization. To them we owe many articles of our daily life, such as bread and cheese, pickles and sauerkraut, linen towels and hemp ropes, explosives and automobile lacquers, plastics and penicillin. But we owe them more than merely the supply of things and materials which make life pleasant. They multiply in our intestine and continuously provide us with certain vitamins as long as we live. They are necessary for our existence. And that is not all. There could be no life on earth without microbes. They decompose all dead plants and animals and reduce them to "dust" from which all new life originates. Through millions of years, they have been an indispensible link in the rotation of elements in nature. They are a more important part of creation than man, for life on earth could very well continue without us.

Among the many good and harmless animals on earth are a few very dangerous ones, like rattlesnakes or scorpions; among the greatly varied vegetation are such plants as poison ivy and nightshade. It is to be expected that among the very large bacterial population of the world, some species exist which are harmful to man. These disease bacteria are relatively rare. Though they represent only a very small fraction of the bacterial population of the earth, they have received much more attention than the good ones, and many popular books have been written about them. That is not surprising, for it is an odd trait of human nature that stories about thieves, robbers and murderers are in greater demand than stories about good, law-abiding citizens. We can certainly be proud of the achievements in fighting bacteria responsible for disease, and it is fortunate that good writers have handled that subject admirably. But after all, the many good bacteria deserve some consideration, too,

and the need for an enthusiastic picture of the other side was felt. This book tries to give that picture.

Many friends have assisted in assembling and arranging the material. I am under obligation to all those who provided the illustrations which help so much to make the text more easily comprehensible. Each illustration gives credit to its author. I am under even greater obligation to my wife, my son Hermann, and my daughter Margarete, for their continuous suggestions and constructive criticisms, for their help with drawings and with photographs.

OTTO RAHN.

ITHACA, N. Y.
November, 1944

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Bacteria are omnipresent. It is easy to keep cats and dogs out of the house; rats and mice get in more readily. Flies and mosquitoes are quite a problem, to say nothing of cockroaches and ants, but to keep bacteria out is plainly impossible. They are on our skin and our fingertips by the thousands. Even when a good scrubbing with soap has removed 99% of them, some remain alive, hiding deep in the pores where disinfectants penetrate but very slowly. As soon as we put our carefully washed hands on the table, a pencil, a door knob, a coat, they are infected again with numerous bacteria. These bacteria are harmless enough, but they are there, unavoidably. We simply cannot escape them.

Naturally, we are not quite satisfied that they are small, we want to know *how* small they are. Although Leeuwenhoek discovered some of the larger kinds more than 250 years ago with lenses that magnified only about 160 diameters, a magnification of at least 500 is necessary to see the smaller kinds. As a rule, the bacteriologist, in routine work, uses a magnification of 1000.

Magnifications are usually recorded by the apparent increase of one dimension. But we see two dimensions, namely the length and the width of bacteria. In a microphotograph, the length is enlarged 1000 times, and the width is also magnified 1000 times, so that the area which we see is actually one million times enlarged. If we could see the third dimension too, if we could get a conception of the volume, we would see it enlarged one billion times. Our microscopes are really quite powerful magnifiers.

All popular texts tell you that the average bacterium is about one-twentyfive thousandth of an inch in diameter. This is correct, but does not assist our imagination. We cannot form a mental picture of such a small measure. The bacteriologist does not measure with inches and fractions of an inch, but he uses the metric system. A millimeter is one-thousandth of a meter (which is about the same as a yard), and a micron is one-thousandth of a millimeter. The

micron, usually abbreviated by the Greek letter μ, is the yardstick of the bacteriologist. Most bacteria are a little less than one micron (1 μ) in diameter. This new measure does not help our imaginations either. We can make comparisons only with something we know well. A woman's hair is about 60 μ thick. Therefore, 60 bacteria side by side would be about as wide as the diameter of a hair. One thousand of them would be as wide as the thickness of a dime.

Although very small, bacteria are not all of the same size. Some relatively large species have a diameter of 4 μ while

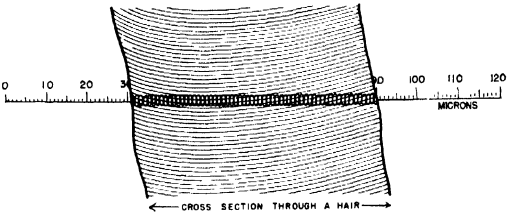


FIG. 1. Sixty bacteria can be placed side by side across a woman's hair.

some tiny bacteria measure only 0.2 μ. This contrast is similar to that between a mouse, less than one inch thick, and a cow measuring two feet across. The *length* of bacteria varies still more. Some are as long as they are wide while others are like threads, a hundred times as long as their diameter.

These measurements refer only to the "usual" types of bacteria, to the kinds which we usually keep in pure cultures in our laboratories. But Nature provides other groups which cannot be grown in pure culture. Among them, especially in the group of sulfur bacteria, we find some that are real whales in comparison with the "usual" types. *Beggiatoa mirabilis* forms threads of 15 μ diameter, and among the spherical kinds is one, *Thiophysa*, with a diameter of 25 μ.

The very small size of the bacteria is of considerable

importance to *them*, and to *us*. It was stated before that our imagination cannot keep step with measurements of the above kind. Here is an example: Sour milk contains a billion bacteria per cubic centimeter, or a trillion per quart. This statement has sent some newspaper writers to wondering whether the nutritive value of milk is not entirely due to the bacteria in the milk. But they are wrong as usual, as a simple calculation reveals. Milk is made sour by *Strepto-*

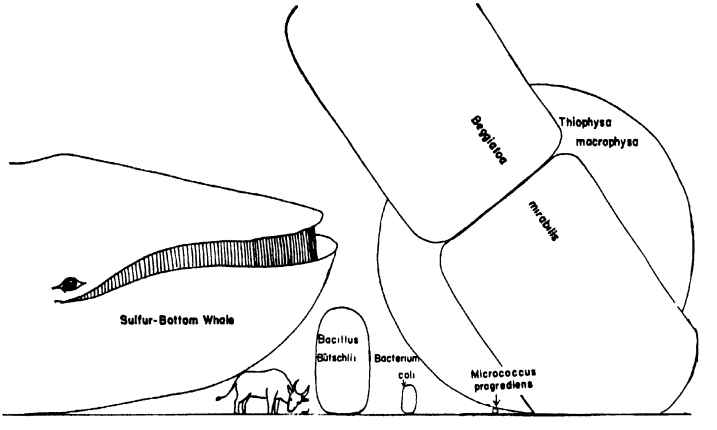


FIG. 2.

Largest, medium, and smallest mammals (do not overlook the mouse). Largest, medium and smallest bacteria.

coccus lactis which is very nearly spherical, with a diameter of about 0.8 μ. The volume of a trillion of these bacteria is 2.3 cubic centimeters. A quart of milk has nearly 1,000 cubic centimeters, and all the sour milk bacteria amount to scarcely one-fourth of one per cent of the milk. They are such an insignificant part that the milk analyst pays no attention to them.

But their products are not insignificant. This large number of bacteria, which weighs so little, makes the milk so sour that it becomes thick, and the casein is separated which we eat as cottage cheese. The analyst cannot afford to neglect the *products* of bacterial action.

Bacteria are so small that they cannot be removed from liquids by ordinary filters because they pass right through the pores. Dry bacteria can be kept out by dry cotton, but in a liquid they are not stopped at all by cotton. Special filters have been constructed to take bacteria out of liquids where they are not wanted, e.g. out of serum or antitoxin, or, to think of something more pleasant, out of fruit juices, or beer

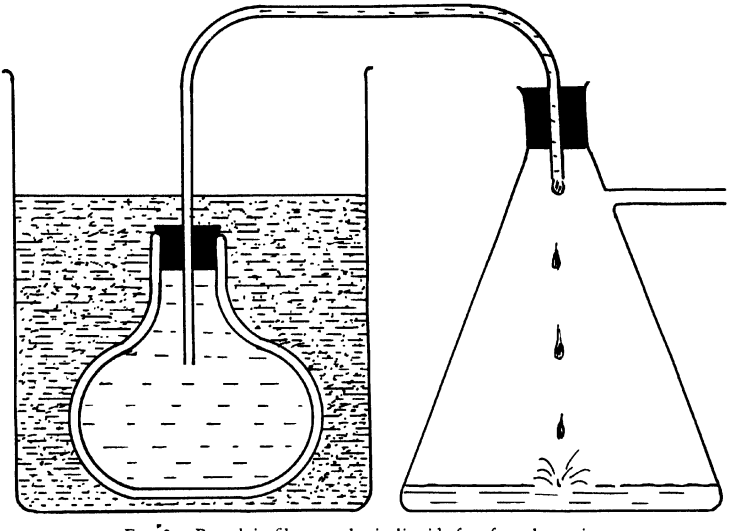


FIG. 3. Porcelain filter to obtain liquids free from bacteria.

or wine. The older type filters are made of unglazed porcelain, or of infusorial earth. They are designed for small quantities and are commonly used in the laboratory. They are either "candle" shaped or bulbous to offer a larger filtering surface. Figure 3 shows how they are used. The bulbous filter is completely submerged in the liquid, closed by a stopper through which a glass tube is pushed which ends in a suction flask. When the air is pumped out of this flask, the liquid will be drawn slowly through the unglazed porcelain of the filter and will drop into the flask. Other laboratory filters are shown in Fig. 38 of Chapter Seven.

For commercial filtration, large filters are constructed which can deliver several thousand gallons of sterile liquid per hour. They are used in the beverage industries.

Smaller than bacteria are the viruses which some biologists consider to be organisms, while others believe that such simple things, which consist of only one molecule and which

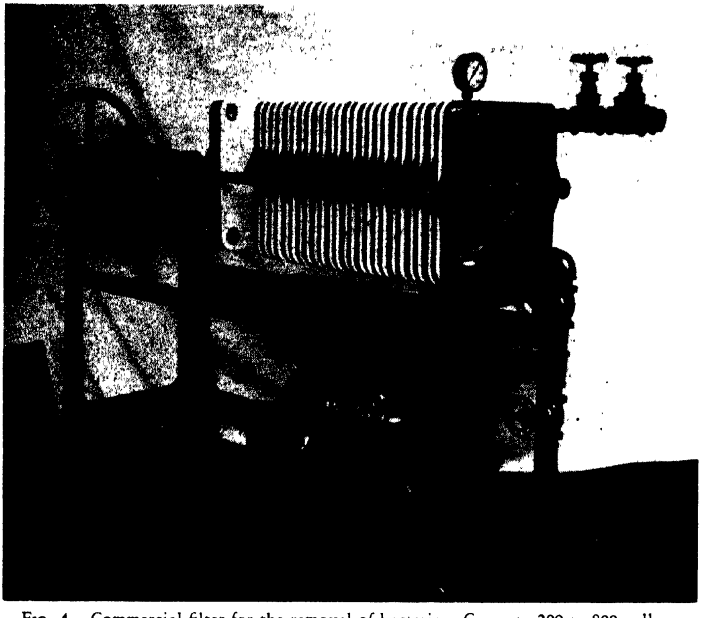


FIG. 4. Commercial filter for the removal of bacteria. Capacity 200 to 800 gallons per hour. (Courtesy of F. R. Hormann and Co., Brooklyn 2, N.Y.)

form crystals like salt and sugar, cannot be called living. Viruses are "ultra-microscopic," invisible even with the best microscopes. But shadow pictures have been obtained with the newly discovered electron microscope which makes even single protein molecules visible. Our picture shows the electron photograph of a virus which attacks bacteria in the same way as the smallpox virus attacks man. This virus is called bacteriophage (bacteria eater), and it destroys the bacteria completely although it is very much smaller.

Other microbes are larger than bacteria, namely the protozoa, the yeasts and the molds. The protozoa will not be discussed in this book, for they are animals whereas all other microbes are plants. Protozoa are of very limited usefulness to man, directly or indirectly, while the yeasts have been domesticated for thousands of years, mostly for alcohol production, and the molds have also been harnessed to do various jobs for us. These two groups will be dealt with in Chapter Four. The present chapter is reserved for the discussion of bacteria.

FIG. 5. Electron Microscope Photograph of *Bacterium coli* and of a virus (bacteriophage) represented by 5 black spots, attacking the bacterium (20,000 times magnified). (Courtesy of Journal of Bacteriology; photo by S. E. Luria, M. Delbruck and T. F. Anderson.)

Bacteria are not only very small but also very simple in form, so that they do not look at all impressive under the microscope. They are not nearly as attractive as protozoa which show the most complex forms, nor as beautiful as the diatoms with their graceful outlines. Even if bacteria could be magnified much more than our microscopes permit, we would not see more detail. They have no mouth and no visible digestive system. They can use only food which is dissolved in water. If food is not soluble, such as fat or starch, the bacteria can secrete certain substances, called enzymes, which dissolve these foods. The dissolved food diffuses into the bacteria, it is digested and decomposed within the bacterial body, and the products are excreted again by diffusing into the surrounding water.

Of this important digestive mechanism, nothing is visi-

ble. We can get shadow pictures of bacteria now by means of the electron microscope which magnify as much as 20,000 times and more, but they do not reveal any more detail. We have to admit that as far as looks go, bacteria are unin-

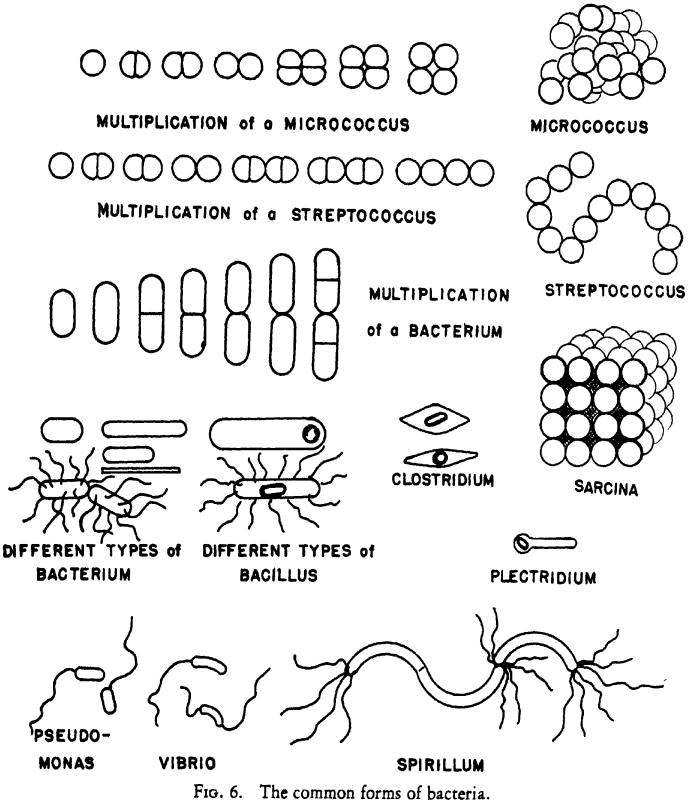


FIG. 6. The common forms of bacteria.

terestingly monotonous. They certainly make up for this lack by an astonishing variation in activities, but under the microscope, we see only three main types: namely rods, spheres and screws. We notice some swimming about while others never move. They may be large or small, plump or thin, rounded off at the ends like a sausage or cut off sharply

like a cigarette. That is nearly all the variety of form that we can observe in bacteria, and as there are several thousand different species, it is obvious that we cannot distinguish all of them from their pictures under the microscope. No bacteriologist can differentiate, by the use of the microscope alone, between the typhoid bacterium and the harmless colon bacterium of which several billions live in our intestine. Very few could distinguish the streptococcus of blood poisoning from the *Streptococcus cremoris* which gives the special flavor to sour cream butter. Their distinction lies in their biochemical activities. The colon bacterium ferments sugar to gas, the typhoid bacterium makes no gas. The milk streptococcus makes more acid than the pathogenic streptococcus, and ferments different sugars. This will be discussed in more detail in a later chapter.

Since we must have different names for different bacteria, we name them by their forms. The rods are called *Bacterium* or *Bacillus;* both words are diminutives of the Latin word for rod which is *baculum.* The spherical bacteria are called *Coccus* which is Greek for ball, and the screw-shaped species are called *Spirillum.* There are further subdivisions some of which are commonly known, and some are so rare that even most bacteriologists cannot remember them. If you get the opportunity, ask a bacteriologist to tell you what a *Mycoplana* is, or a *Rhabdomonas.* The chances are hundred to one that he has never heard of a bacterium by that name. Nor will they be explained here because they are very rare and very unimportant. But a few of the more important species should be mentioned here to give a general picture of the forms of bacteria.

The entire group of bacteria is called *Schizomycetes,* which means fungi or molds which split in two when multiplying. They are subdivided into orders, and the orders are subdivided into families. Only a few of the many families interest us here, namely the *Bacteriaceae* which are rod-shaped, without spores; the *Bacillaceae* which are rod-shaped, with

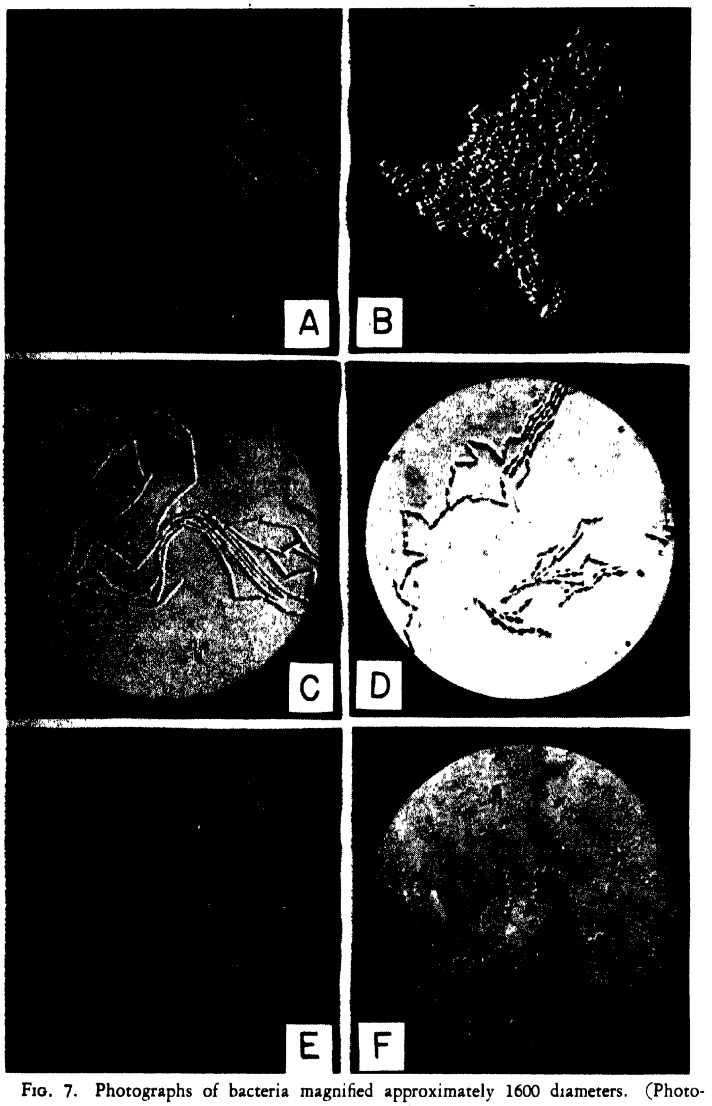


FIG. 7. Photographs of bacteria magnified approximately 1600 diameters. (Photographs by Dr. J. J. Schmidt, Hartenholm in Holstein.)

A. Streptococcus cremoris, producing the flavor in sour cream.
B. Sarcina lutea, a common soil and water bacterium.
C. Bacillus cereus, a soil bacterium; young cells.
D. Bacillus cereus, old cells showing spores.
E. Bacterium coli, inhabitant of human intestine.
F. Spirillum rubrum, a rare water bacterium.

spores; the *Coccaceae* which are spherical, and the *Spirillaceae* which are screw-shaped.

Spores are round or oval granules which appear in the cells of some species when they get old. The rest of the cell then dies, but the spores survive, and they are very tough as will be shown in Chapter Six. Most of them can survive boiling for a while. Freezing, drying or starvation does not kill them. Their resistance to disinfectants is also very

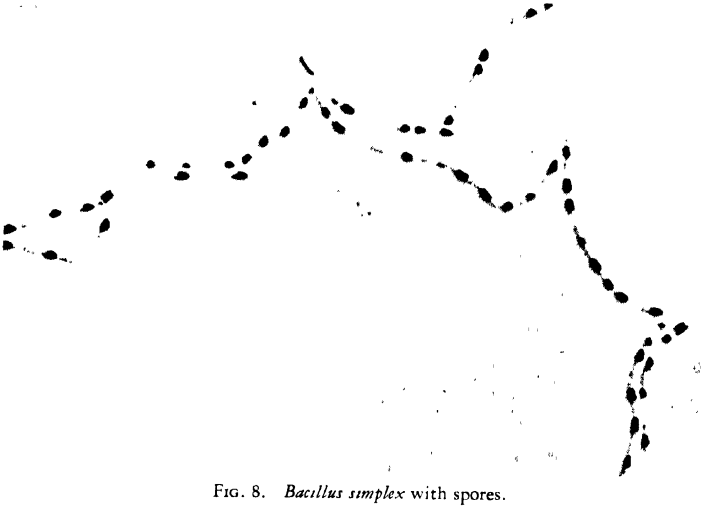


FIG. 8. *Bacillus simplex* with spores.

great. When these spores are transferred to a fresh medium, they germinate, and the young cells multiply like all other bacteria. This is called the vegetative stage. When they get old, each cell makes one spore. These spores cause us a lot of trouble, for instance in the canning of foods; but they are a great help to the bacteria for weathering adverse conditions. Less than 10 per cent of all species can make spores.

The speed with which some bacteria can swim about, looks quite amazing under the microscope while other species simply flounder about lazily. The speed which we

see is magnified, of course, and what appears like an inch per second is really only an inch in 1,000 seconds, or four inches per hour.

However, the speed may be considered relatively, as the time in which the bacterium can move out of its own environment, or as the speed in relation to its length. If we take the fair speed of 1 inch per second for a long bacillus, that is 25 μ of actual movement per second for a cell of 4 μ in length, or 6 times its length in a second. The corresponding relative rate for a race horse would be 30 miles per hour.

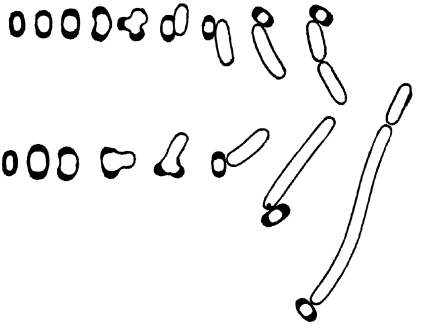


FIG. 9. Successive stages of the germination of bacterial spores. (From Lafar, Technische Mykologie.)

Bacteria move by long, but very thin appendages which are invisible even by high magnification. The cause of motility of bacteria was doubtful until in 1890, when Loeffler tried to dye them in the same way as cotton is dyed, namely by using a mordant or fixative. After such treatment, the appendages retained the dye and could be recognized as long, thin hairs. Some types of bacteria have only one such organ, called flagellum (Latin for whip) attached to the pole of the cell, others have many flagella all over the body. The electron microscope shows flagella without staining. Figure 7 is a single cell, ready to divide, of Azotobacter, a very useful bacterium.

After we have realized that bacteria have only three fundamental forms, and that some of them have spores, and

some of them have flagella, it might be well to have an introduction to some of those species which we shall meet again in some of the later chapters.

FIG. 10. *Azotobacter vinelandii*, with flagella. Electron Microscope photograph, 27,000 times magnified. (Courtesy of Dr. A. W. Hofer, Agr. Experiment Station, Geneva, N.Y., and R. C A. Laboratories, Princeton, N.J.)

The *Bacteriaceae*, rods without spores, are the most numerous group. Many genera are distinguished. *Pseudomonas* is the group with a single polar flagellum. *Lactobacillus* means a slender rod which ferments sugar to lactic

acid. We will meet them in various food fermentations. Also to this family belong a number of queer soil bacteria, with which we will get acquainted soon, like *Hydrogenomonas*, *N trobacter*, *Azotobacter*, and also the vinegar bacteria, called *Acetobacter*. The large group of intestinal bacteria, some of which cause intestinal diseases, e.g. typhoid and dysentery, have been given some fancy names like *Escherichia*, *Eberthella*, *Salmonella*, etc., but for our purpose, the simple, old term *Bacterium* is good enough. Outstanding among these is *Bacterium coli*, one of the most common bacteria on earth and which is a regular and necessary inhabitant of the intestines of all larger animals, including man.

Among the *Bacillaceae*, the sporeformers, only two genera are distinguished. *Bacillus* means an "aerobic" sporeformer which must have air to live, and *Clostridium* means an "anaerobic" sporeformer which lives only in the absence of air, and to which oxygen is poison. There are many different species in each group. They are of great importance, and will be discussed in many other chapters.

Among the *Coccaceae*, three groups are distinguished according to their mode of division. Those that divide in one plane only, and produce chains of little balls, are called *Streptococcus*. There are a few pathogenic or disease producing species among the streptococci, but there are also some very useful ones such as those which produce the aroma of butter, the acid which is necessary for cheese making, and those which change cabbage to sauerkraut.

If the cell division is in two alternate planes, squares of four cocci are produced. This is characteristic for the genus *Micrococcus*. If division takes place successively in three planes, little packets originate which are called *Sarcina*. Often, the cells break loose as soon as they are formed, and simply produce a mass of spheres without any regularity. They are called *Staphylococcus* which means grape balls because they resemble a grape cluster.

The Spirillaceae have two genera: *Spirillum* means large

screws, sometimes consisting of several revolutions, with a tuft of flagella at the end; and *Vibrio* represents smaller, only slightly curved rods with but one flagellum.

The complete list of families and genera is much longer, but this is not a textbook. In the course of our discussion, other names will turn up occasionally, but they need no special introduction at this time.

Chapter Two

THE DISCOVERY OF BACTERIA

Once upon a time, or to be more precise, 300 years ago, there lived in the quiet town of Delft, in Holland, a man by the name of Anthony van Leeuwenhoek. He had owned his haberdashery store ever since he was 22 years old. He was a man of many talents, for the city appointed him Sheriff's Chamberlain when he was 28 years; he passed the surveyor's examination at 37 years; and finally became the wine gauger of the city. These various duties could not have consumed all his time, because he became famous through his hobby, which was the study of nature with powerful glass lenses in the manufacture of which he had developed a remarkable skill. Even the compound microscope invented some 70 years earlier by the Jansens, father and son, at Middelburg, did not magnify as highly as Leeuwenhoek's home-made lenses. He was not a scientist, and never pretended to be one. He considered himself a layman, and in 1673, he wrote to the Secretary of the Royal Society of London:[1]

I have oft-times been besought, by divers gentlemen, to set down on paper what I have beheld through my newly invented *Microscopia:* but I have generally declined; first, because I have no style, or pen, wherewith to express my thoughts properly; secondly, because I have not been brought up to languages or arts, but only to business; and in the third place, because I do not gladly suffer contradiction or censure from others. This resolve of mine, howeve, I have now set aside, at the entreaty of Dr. Reg. de Graff; and I give him a memoir on what I have noticed about mold, the sting and sundry little limbs of the bee, and also about the sting of the louse. . . .

I beg you, therefore, and those gentlemen to whose notice these (drawings) may come, please to bear in mind that my observations and thoughts

[1] All quotations and drawings concerning Leeuwenhoek are taken from the book: Anthony van Leeuwenhoek and his "Little Animals" by Clifford Dobell, published on Leeuwenhoek's 300th birthday by Bale Sons and Danielson, London, 1932.

are the outcome of my own unaided impulse and curiosity alone; for, besides myself, in our town there be no philosophers who practise this art; so pray take not amiss my poor pen, and the liberty I here take in setting down my random notions.

This "unaided impulse and curiosity" drove him to use his lenses for the study of all kinds of materials, and he became quite a noted figure when, in 1675, he discovered, in rain water, rapidly moving animals which were so small that the most famous microscopist of his time, Robert Hooke, in England, could not confirm this until two years later. Still tinier living things he saw in 1676 when he tried to find, with the microscope, the cause of the hotness of pepper. There can be little doubt that the smallest rod-shaped beings he observed in rotting pepper were bacteria.

In 1679, Leeuwenhoek was made a member of the Royal Society of London to which he had reported the results of his findings for several years. In 1680, he described in one of his reports the microscopic appearance of beer yeast. He did not consider them to be living; to him they were just "*feces vini*," the excretion of wine, a byproduct of the alcoholic fermentation and not its cause. In 1681, he wrote about a new kind of tiny beings found in feces whose nature may be doubtful. But the "little animals" (animalcula) which he found in the tartar of his teeth in 1683 certainly were bacteria, for they were accompanied by a drawing. This is the first drawing ever made of bacteria. He describes them as follows:

T'is my wont of a morning to rub my teeth with salt, and then swill my mouth out with water: and often, after eating, to clean my back teeth with a toothpick, as well as rubbing them hard with a cloth. . . . Yet notwithstanding, my teeth are not so cleaned thereby, but what there sticketh or groweth between some of my front ones and my grinders a little white matter which is as thick as if it were batter. On examining this, I judged that there yet were living animalcules therein. I have therefore mixed it, at divers times, with clean rain water, and also with spittle which I took out of my mouth, after ridding it of air bubbles: And I then most always saw, with great wonder, that in the said matter

there were many very little living animalcules, very prettily a-moving. The biggest sort had the shape of Fig. A: these had a very strong and swift motion, and shot through the water like a pike does through water. These were most always few in number.

The second sort had the shape of Fig. B. These ofttimes spun around like a top, and every now and then took a course like that shown between C and D, and these were far more in number.

To the third sort, I could assign no figure: for at times, they seemed to be oblong, while anon they looked perfectly round. These were so small that I could see them no bigger than Fig. E: yet therewithall, they went

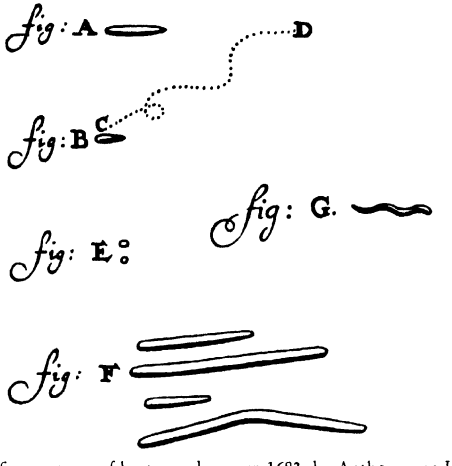


FIG. 11. The first pictures of bacteria, drawn in 1683, by Anthony van Leeuwenhoek.

ahead so nimbly, and hovered so together that you might imagine them to be a big swarm of gnats or flies, flying in and out among one another. These last seemed to me e'en as if they were in my judgement, several thousand of 'em in an amount of water or spittle no bigger than a sand-grain; albeit there were quite nine parts of water, or spittle, to one part of the matter that I took from betwixt my front teeth, or my grinders.

Furthermore, the most part of this matter consisted of a huge number of little streaks, some greatly differing from others in their length, but of one and the same thickness withall; one being crooked, another straight, like Fig. F, and which lay disorderly ravelled together. And because I had formerly seen, in water, live animalcules that had the same figure, I did make every endeavor to see if there was any life in them; but I could make out not the least motion that looked like anything alive, in any of 'em.

While I was talking to an old man my eyes fell upon his teeth, which were all coated over; so I asked him when he had last cleaned his mouth? And I got for answer that he'd never washed his mouth in all his life. So I took some spittle out of his mouth and examined it; but I could find in it naught but what I had found in my own and other people's. I also took some of the matter that was lodged between and against his teeth, and mixing it with his own spit, and also some fair water. I found an unbelievably great company of animalcules, a-swimming more nimbly than any I had ever seen up to this time. The biggest sort (whereof there were a great plenty) bent their bodies into curves in going forwards, as in Fig. G. Moreover, the other animalcules were in such enormous numbers, that all the water seemed to be alive. The long particles too, as before described, were also in great plenty.

The discovery of invisible organisms which move about rapidly and which can be made visible by glass lenses, caused a good deal of excitement among the educated classes of that time. Hooke wrote Leeuwenhoek that his Majesty the King of England had been quite interested in seeing the animalcules. Though Leeuwenhoek never sold any of his microscopes, other makes such as the Jansens' type were sold in Holland, England, France, Germany and Italy. Leeuwenhoek became a famous man among the philosopher-naturalists of his time, and the newly discovered "world in a water drop" remained for more than a century the object of philosophical discussions and caused learned and religious men to wonder why the Lord had created such tiny creatures. Cotton Mather who owned one of the first microscopes in America, wrote about them in "The Wonderful Works of God Commemorated: A Thanksgiving Sermon" in 1690 as follows:[1]

And the *Little* Things which our Naked Eyes cannot penetrate into, have in them a *Greatness* not to be seen without *Astonishment*. By the Assistance of *Microscopes* have I seen Animals of which many Hundreds would not Aequal a Grain of Sand. How Exquisite, how Stupendous must the Structure of them be! The Whales . . . methinks . . . are not such Wonders, as these minute Fishes are.

[1] Quoted from an article by F. T. Lewis in Scientific Monthly, vol. 57 p. 249, 1943.

Shortly before Leeuwenhoek's discoveries, The Jesuit Pater Athanasius Kircher had found by means of a microscope that all putrefying matter contained "worms," and he observed them later also in blood and pus. As his lenses did not magnify more than 35 diameters, he could not have seen bacteria, but probably protozoa of the putrefying substances, and red and white blood corpuscles in the blood and pus. These were not known at that time. Kircher's great imaginative powers constructed from these few observations a theory that contagious diseases were caused and spread by these "worms." This theory of a "contagium animatum" came back to life through Leeuwenhoek's discoveries, and around 1720, after an epidemic of bubonic plague in France, the fear of "worms" became such an obsession with the population, that some of the more conservative doctors tried to appease the public by ridiculing the idea in satyrical books.

When Linnaeus, the great Swedish biologist and father of modern taxonomy, made his famous classification of plants and animals, he was in doubt about the microscopic organisms. In his 10th edition, 1758, he placed them under *Vermes*, worms. In the 12th edition, he groups them in one genus, really one species, *Chaos infusorium*. He did not study these organisms, but considered it possible that they might cause certain diseases. At about this time, in 1761, appeared the book of a Viennese physician, M. A. Plencig, who developed the theory that contagious diseases were caused by microscopic animals. He had studied putrefaction and fermentation and drew analogies between these processes and contagious diseases which were quite convincing.

Soon after Linnaeus, the first attempts were made to bring order into Linnaeus' "Chaos," according to the morphological properties of these animalcules, or "microscopiques" as the French called them. The most important, well illustrated book is that by Otto Friedrich Müller of Copenhagen entitled *Animalcula infusoria fluviatilia et marina*, 1786.

Naturally, it contains descriptions of protozoa as well as bacteria, but all later attempts at classification are based on his thorough investigation.

Müller had grouped the infusoria under the general chapter heading of worms in 1773, but in 1778, he mentions that some of the infusoria appeared to him as intermediate

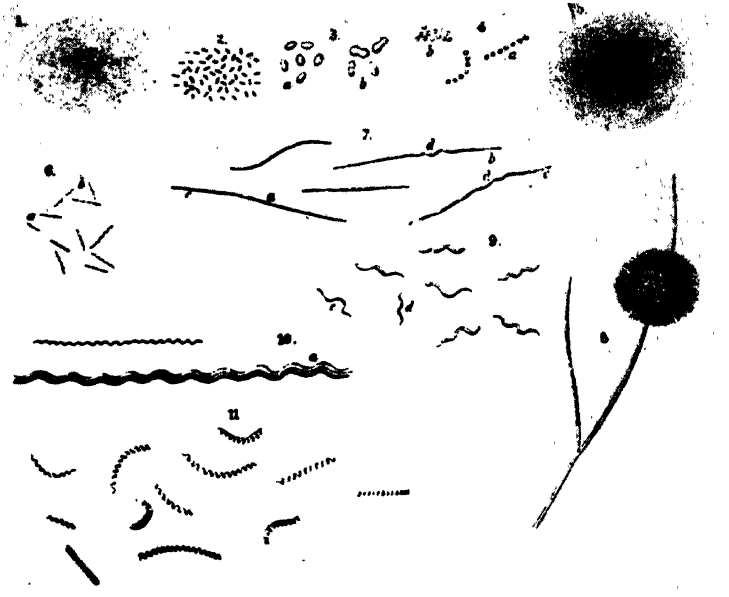


FIG. 12 Some drawings of bacteria, by Otto Friedrich Müller, from his book *Animalcula Infusoria Fluviatilia et Marina*, 1786.

forms between plants and animals. He was not thinking of molds whose plant nature had never been doubted, but of certain threadlike algae, the confervae. In his classification, he uses the genus names *Proteus* and *Vibrio* which are still used in bacteriology, though with different definitions. He distinguished between *Vibrio bacillus* and *Vibrio spirillum*.

The word *Bacterium* was not used until 1838 when Ehrenberg in Germany wrote his book on Infusoria and thereby revived the interest in the "animalcules" which had become

almost forgotten because of the big world affairs of the French revolution and the Napoleonic wars. Ehrenberg distinguished between *Bacterium*, *Spirillum*, *Vibrio*, *Spirochaete* and *Spirodiscus*. The terms *Bacillus* and *Micrococcus* were finally introduced by Cohn as late as 1872.

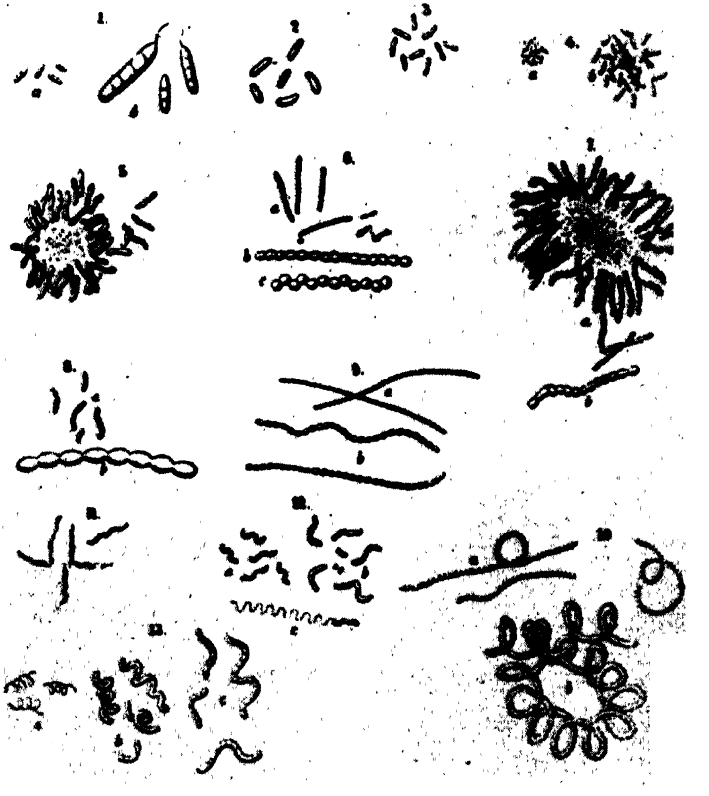


FIG. 13. Some drawings of bacteria from Ehrenberg's *Infusoria*, 1838.

More important than these purely descriptive attempts at classification were the observations by Cagniard Latour in France and by Schwann in Germany in 1837 that the yeast is a plantlike organism whose budding and growth during fermentation can be plainly seen under the microscope. (By

this time, achromatic compound microscopes with considerable magnifying power had been constructed.) These two investigators had come, independently of each other, to the conclusion that yeast is not a *by-product* of the fermentation (the old alchemists had called it *faeces vini*) but the *cause* of fermentation. To this great step in the understanding of microbial activity must be added the observations of Kützing who not only studied the alcoholic fermentation, but could prove that the formation of vinegar was the result of the activity of the "mother-of-vinegar," the slippery membrane developing on the surface of a vinegar cask, and he recognized this membrane as an organism of vegetable nature.

These facts which are now known to every highschool student, were so revolutionary 100 years ago that such an outstanding man as the great German chemist Justus von Liebig absolutely refused to accept them. To him, fermentation and putrefaction were purely chemical processes, and he gave vent to his scorn of these newfangled views in a sarcastic parody published in his own scientific magazine in 1839. He pretended to have seen, through a new type of microscope, animals hatching from the yeast which represents the eggs of these animals.

> The form of these animals is different from that of any of the known 600 species, they resemble a Beindorf distillation apparatus. . . . Teeth and eyes could not be observed, but one can plainly discern a stomach, intestine, anus (as a rose-colored point) and the organs of urine secretion. These animals swallow the sugar of the solution, one can plainly see its arrival in the stomach. It is digested instantaneously, and the digestion is most definitely visible by the elimination of excreta. In one word, these infusoria eat sugar and they eliminate alcohol through the intestine and carbon dioxide through the urinary organs. The bladder has the shape of a champagne bottle. . . . The fusel oil seems to be secreted from the surface of their skins through a kind of sweating process.

It is not surprizing that the botanists could not hold their own against a genius like Liebig, because the main dispute centered around the cause of certain chemical reac-

tions, usually described as fermentations, but including such processes as putrefaction. To counter the bold statement by Liebig that "causes cannot be seen through a microscope," a man well versed with all detail of chemistry was needed. It took nearly twenty years before the botanists Schwann and Kützing found an ally who was a match for Liebig, namely the French chemist Louis Pasteur.

Pasteur began his studies in 1857 with the souring of milk. It was known then that milk soured because the milk sugar, lactose, was changed to lactic acid by means of a fermentation. All fermentations were believed, according to Liebig's theory, to be brought about by some protein, the so-called ferment, which had the peculiar ability of changing certain substances into certain others. Pasteur found that heated milk does not sour by itself, but can be made to sour again by transferring a tiny trace of sour milk. The souring was brought about by an exceedingly small globular kind of "yeast," quite different in size from the wine and beer yeasts. This new "yeast" could grow in a beer yeast extract to which sugar and chalk had been added. Pasteur concluded that it was not the milk protein which changed the sugar to acid; but that the fermentation was caused by this new yeast which needed the sugar in its metabolism; and that the lactic acid was a metabolic product of the new "yeast."

In the following years, Pasteur studied other fermentations in a similar systematic way. The solution to be fermented was first heated, and then, it did not decompose. When a trace of the unheated fermenting material was added, the organisms multiplied and fermentation started.

Pasteur used the microscope as well as the testtube, but the botanical or zoological classification of the organisms did not interest him. To be sure, he was greatly puzzled when he found in 1863 that the butyric acid fermentation of sugar was caused by organisms which were very actively motile. He felt that he could not call them yeasts, nor plantlike organisms. Since they were motile, he regarded them as

animals. So he called them "animalcules infusoires" or "vibrios." But he did not worry about this. "Whether the progress of science will consider this vibrio as a plant or an animal, does not matter much at present. To live without air, and to be a ferment, this represents two properties which separate this vibrio from all lower organisms of both kingdoms."

The conception of Pasteur that each fermentation is caused by the metabolism of a certain species of microorganism is still essentially correct at the present time. At last, nearly two centuries after the discovery of bacteria, mankind had finally found out the cause of fermentation and began to realize why the Lord had created such tiny animalcules. Although Pasteur's views were doubted and disputed by many of his contemporaries, his explanation of fermentations and putrefactions withstood all criticisms. He had proved beyond any doubt that fermentation and putrefaction were caused by microscopically small organisms, bacteria or yeasts or molds; that the decomposition was part of their metabolism, comparable to the digestion and respiration of animals; and that each group of bacteria produced a different type of decomposition. Frequently, one type of fermentation followed another as e.g. the vinegar follows the alcoholic fermentation, and the product of yeast metabolism, the alcohol, is the main food of the vinegar bacteria.

The analogy of microbial decompositions with digestion and respiration of animals was not perfect. Animals oxidize the digestible food almost completely to carbon dioxide and water while the endproduct of bacterial activity is often some organic compound like the lactic acid of sour milk, the acetic acid of vinegar, or the alcohol of the yeast fermentations. Pasteur realized that in many fermentations, air or oxygen was not needed. The wine and beer yeasts, and the bacteria which make milk sour, can live and ferment without air. The bacteria which produce butyric acid not only live without air, but air, i.e. oxygen, is a very strong

poison for them. Pasteur's statement: "Fermentation is respiration without air" expressed this relation to oxygen in a general way.

After Pasteur had thus changed fundamentally the conceptions of fermentation and of the importance of microorganisms, he started his investigation on the contagious diseases of silkworms which at that time constituted an important industry in France. From that, he went into the study of anthrax or spleenic fever. This drew him more and more to the investigation of contagious diseases of men and animals, although he still published papers on the "diseases of wines" and his well-known studies of the beer fermentation.

By 1870, Pasteur's experiments had become generally known, and his conclusions had weathered the storms of all criticisms. Many of the younger chemists and biologists recognized the new meaning of bacterial activity and used the fundamental ideas and methods elaborated by Pasteur to study the many problems in applied and theoretical sciences which might be of microbial origin. By 1890, many laboratories of bacteriology were turning out investigations of the most widely differing subjects, all over Europe, from England to Russia.

A new science had been born. While the medical side of bacteriology was quickly taken up by the medical schools of the world, the non-medical side remained for some time scattered and split up according to the various applications. The brewer did not know what the dairy bacteriologist was doing, and the soil bacteriologist paid little attention to the bacteriological advances in the manufacture of sauerkraut, or in the retting of flax to make linen. Gradually, the larger fields of agricultural bacteriology took on a definite shape, institutes for the fermentation industries developed, and finally, in the first decade of this century, universities began to train general bacteriologists who got a broad view of the various fields of applied as well as theoretical bacteriology.

Pasteur had already clearly recognized that bacteria were

the scavengers on earth, whose main object it was to decompose all dead organic matter. He had realized that bacteria completed the cycle in the rotation of carbon in nature which his great antagonist, Justus von Liebig, had revealed by his discovery that plants produce their organic matter not from the humus of the soil, but from the carbon dioxide of the air. He had laid the cornerstone for the application of bacteria in the food industries, and on these foundations, a science of general and applied bacteriology has been built up during the last 60 years which will be discussed in the following pages.

Chapter Three

AMAZING APPETITES

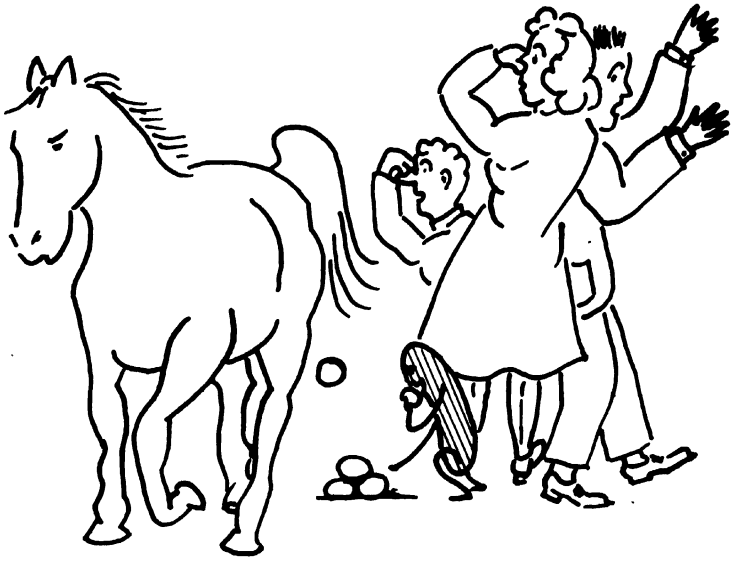
The appetites of bacteria are amazing in respect to quality as well as to quantity. The amounts of food they can digest are almost unbelievable, and the kinds of food that *some* bacteria live on are simply horrifying, but they are in keeping with the primary object of bacteria on earth: to decompose all dead organic matter. To be sure, we too decompose various kinds of dead organic matter by digesting them, but they are quite select, such as T-bone steak with mushrooms and apple pie. Bacteria, however, are meant to decompose *all* kinds of organic matter, and that is quite a different story. Some of the diets of bacteria are strange, and some are really revolting to us.

It must be admitted that in the laboratory, bacteria are usually fed on a meat broth which is quite good. For some purposes it is jellied with gelatin or agar. Often, a little sugar is added which may not appeal to *our* taste, but many bacteria and all yeasts love this combination. Many bacteria like milk. Then, for yeasts, we keep cider and grape juice. Some disease bacteria want blood, like the "blood-loving" *Hemophilus influenzae* which was believed to cause influenza before the influenza virus was discovered, and the *H. pertussis* which brings about whooping cough.

The bacteria which most laboratories keep in stock represent a rather onesided selection. Many very important species are found in only a few specializing laboratories.

One group of bacteria decomposes cellulose, the main constituent of paper and wood. Some of these bacteria cannot feed on anything else. They thrive on wet filter paper or cotton with a little nitrate and phosphate. Ropes and fish nets are to them dead organic matter of considerable attraction which must be destroyed. The nitrate bacteria

are very queer because they do not use any organic matter whatever. Sugar and meat broth kills them. They live on ammonia. They oxidize ammonium salts like we oxidize sugar and fat, and the result is nitrate. (This goes in two steps as will be shown in Chapter Nine.) Besides these, we find in soil several species of *Hydrogenomonas* which can digest hydrogen gas. These remarkable organisms resorb hydrogen



Amazing appetites.

and oxidize it, and that is their way of respiration. There is a lot of energy in hydrogen, but no other organisms can digest it. These bacteria can live and multiply with no other food but hydrogen gas, oxygen gas, carbon-dioxide gas, and some phosphate and nitrate dissolved in water. Then, there is the *Methanomonas* which uses methane, i.e. marshgas, instead of hydrogen; and the *Carboxydomonas* which lives exclusively on carbon monoxide which is so deadly to all mammals.

Hoping that by now, the reader is prepared for almost

anything, we can mention the very large group of "sulfur bacteria" called the *Thiobacteriales*, with over a hundred different species, which live exclusively or nearly so on hydrogen sulfide, that evil-smelling gas which is usually described as typical of "rotten eggs." This gas is produced wherever animal or plant matter is rotting. To these sulfur bacteria, this gas which dissolves readily in water is the source of energy. They use it as we use sugar. They may oxidize it incompletely, and produce sulfur which they deposit as reserve material in their body as we deposit fat when we eat plenty of sugar. In times of scarcity of hydrogen sulfide, the bacteria use the stored sulfur and oxidize it completely, to sulfates.

If we try to think of other organic matter on earth to be decomposed besides the common proteins, fats and carbohydrates, there is hair, horn, hoofs and nails, consisting of a mixture of very indigestible substances. Well, we all know that they disappear slowly, but completely in buried animals before the bones have disappeared. Only bacteria could have accomplished this destruction. Among the indigestible substances belongs the chitin skeleton of the insects and crabs on which certain bacteria are specializing.

Then, there is resin, and wax, and rubber, all of them natural plant materials which are decomposed slowly, usually by bacteria specializing on those compounds for their daily diet. One of the most difficult things to decompose is lignin which is an important part of wood and woody parts of all plants. The higher fungi, i.e. the mushrooms, can decompose it fairly well, and the edible mushroom as well as the wood-destroying fungi attack lignin and also cellulose. Ordinarily, it is the part of plants which is decomposed last, and the humus of the soil consists, to a considerable part, of lignin in various stages of decomposition.

The above menus may give the impression that bacteria are very onesided in their food. That is true for some, but not for all species. It is true for the nitrate bacteria which

insist on mineral food, and for most sulfur bacteria which can take no other food except that horribly smelling hydrogen sulfide gas. Only a few bacteria can decompose cellulose, and some of these do not seem to be able to digest anything else; but other species of the same group thrive on starch and on sugar. Queer is the Hydrogenomonas which can live on a strict diet of hydrogen, oxygen and carbon dioxide and again grows abundantly on meat broth without any hydrogen.

Quite different is the appetite of the colon bacteria which, as regular inhabitants of our intestine, naturally depend upon the very varied diet which we eat. They are very adaptable. They can live and multiply with nothing but ammonium acetate besides the mineral salts needed, or on ammonium chloride and sugar. But they also grow well in broth without sugar, and even in a solution of a single amino acid, e.g. asparagin. Hay infusion also is an excellent medium for them. One important part of our food is however indigestible for them, namely fat. It is rather surprising how few bacteria can attack fat.

Yeasts can get along without sugar, but are not very happy under such a restricted diet. Fat is of no food value for yeast.

As far as a mixed diet is concerned, the molds hold the record for variability. All of them do well with sugar or starch; many can use cellulose. They can digest insoluble proteins. Most of them decompose fats, and molds are one of the main causes of rancid butter. They love organic acids like the malic and citric and tartaric acids of fruits, and the lactic acid of sour milk, cheese and sauerkraut. If food is scarce, they can adapt themselves; on damp cellar walls, on rotting wood, on freshly washed moist linen, molds are a common occurrence. They can be frugal if they have to.

Many chemicals are made in factories which are called "organic" by the chemist although no organism can produce them. Some of these are digested by bacteria. The tubercle

bacteria and their relatives can live on paraffin; some bacteria digest petroleum compounds, and one small group even gets away with carbolic acid.

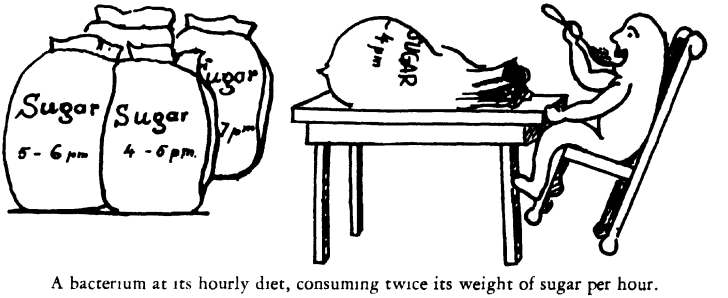
The chapter heading promised that their appetites would be amazing. Really, it seems so only because we are in the habit of considering as "food" primarily those things which we eat ourselves, and which we see the animals eat. If we think of bacteria primarily as scavengers, it is easily realized that all these queer groups mentioned above must exist. They are all indispensible if the organic matter of our earth is to be decomposed.

Now we come to the *quantities* of food that bacteria can get away with. The food intake of bacteria can be easily and accurately measured. If we know the number of bacteria in a culture, and measure the sugar now, and again one hour later, we know how much sugar has disappeared, and how much has been used per bacterium. The actual amounts per cell are of course extremely small, some billionths of a milligram, and such small amounts are beyond the range of imagination. But the bacteria themselves are also very small, and if we record the weight of food consumed in relation to the weight of the bacterium, we have something tangible. A very conservative estimate shows that the lactic acid bacteria consume twice their own weight of sugar in an hour, and continue to do so hour after hour, day and night, until all the sugar is used up, or until so much acid is produced that bacterial activity stops. Strangely, bacteria do not sleep, and they have never been caught napping as long as there was any food around. Yeast uses less than one-half of its own weight of sugar per hour, but some urea bacteria can ferment 100 times their body weight of urea in an hour, changing it to ammonium carbonate.

Bacteria may appear to us as ravenous gluttons until we begin to realize that the rate of metabolism is a question of surface. One quart of a full-grown bacterial culture has about a trillion bacteria, and these have a total surface of

50 square feet. The sugar has to travel an extremely short distance to get from the outside to the center of the very tiny cell, and after it is fermented—which takes less than $\frac{1}{1000}$ of a second—the products of fermentation have to travel only the same short distance again to get out of the cell and make room for more sugar.

How important the amount of surface is to all organisms is evident from the various devises of the larger organisms



A bacterium at its hourly diet, consuming twice its weight of sugar per hour.

to increase their surface. The large flat leaves of the plants permit a rapid exchange of air and carbon dioxide. The very long intestinal tract of animals has the purpose of allowing a large amount of food to be resorbed by the body. The lungs of the human body with their bronchia ending in the alveoles represent a surface of nearly $\frac{1}{10}$ of an acre; such an area is necessary to provide our body with oxygen. That is 4,356 square feet for 150 pounds of body weight, or 30 square feet for every pound. Bacteria have about 1000 times as large a surface. All the bacteria that can grow in one quart of liquid weigh less than one gram, and have a surface

of about 50 square feet. A pound of bacteria, or 452 grams, would have a surface of nearly 30,000 square feet.

Bacteria eat twice their own weight of sugar per hour, or 2 grams per 50 square feet, or 0.4 grams per square foot. If we should eat at the same rate, considering not weight but surface, it would mean $0.4 \times 4{,}356 = 174$ grams of sugar or 6 ounzes every hour of the day, or 10 pounds per day. According to this comparison, bacteria still eat a good deal more than we do. But we get much more good out of the sugar we eat because we digest it more completely as will be shown later. The lactic acid bacteria change the sugar only to lactic acid, and most of the calories of the sugar remain unused in this acid, while we burn up the sugar completely and get all the calories that are in the sugar. The difference is great; we get 26 times as many calories out of each ounze of sugar as the lactic acid bacteria. Therefore, we need only $\frac{1}{26}$ as much sugar as the bacteria, and $\frac{1}{26}$ of 10 pounds of sugar is about 4 ounzes per day. That is perhaps too small an amount; we use more carbohydrate. But it is not very far from the actual total carbohydrate consumption, and considering the inaccuracies involved in calculating bacterial surfaces and human surfaces, the result justifies the reasoning that the basis of comparing amounts of food should be surface and not weight.

Yeasts are larger than bacteria, and a quart of cider at the height of fermentation contains about 6.5 g of yeast with a surface of 60 square feet, or 4,150 square feet per pound. A sugar consumption on the same scale as that of lactic bacteria would be $\frac{2 \times 4150}{30{,}000}$ times the body weight or $\frac{3}{10}$ of the body weight in sugar while the experiment showed about $\frac{5}{10}$.

While these calculations explain the reason for the enormous food consumption of bacteria, the fact remains, nevertheless, that a very small mass of bacteria can decompose a relatively very large quantity of organic matter in a very

short time. That accounts for the rapid souring of milk, the prompt fermentation of cider and grape juice, the bad small of old meat on which no bacteria are visible to the naked eye.

Such calculations help us to understand that the amazing appetites of bacteria are nothing unusual or abnormal. They are simply due to their small size which means a relatively very large surface. The only unusual thing is the smallness of the individual bacterium.

Now that we have discussed the diet of bacteria, we might inquire about their digestion. This reveals an interesting picture. Very few bacteria digest their food in the way the animals do. Animals break down the food by various processes, but the end result is an almost complete oxidation or combustion of the digestible part of the food into carbon dioxide and water. The nitrogen of the protein is not oxidized, but leaves the body in the simple form of urea or of compounds closely related to urea.

Some bacteria can produce a similar complete combustion. The aerobic sporeformers like *Bacillus subtilis*, the bacteria of the *Pseudomonas* group and some of the *Colon* group can do this if the conditions are just right, but usually, that is not the case. It never happens in our customary laboratory cultures, for the very simple reason that the bacteria have not enough oxygen. To be sure, oxygen is one of the easiest things to provide, one-fifth of all the air around us is oxygen. But that is in the air, and the bacteria are in the culture, under the surface of the broth. It is surprizing how very little air gets to them. Oxygen is soluble in water or broth only to the extent of about 7 to 9 parts per million parts of liquid. This can be best visualized by an example. The tubercle bacteria love glycerine more than sugar. They oxidize the glycerine completely to carbon dioxide and water, with no byproduct. For the manufacture of tuberculin, the bacteria are grown in a liquid containing 3% glycerine, or about one ounce per quart. To oxidize this glycerine,

they need 3¼ ounces of oxygen. The amount of oxygen dissolved in a quart of liquid is 0.0003 ounces. No wonder that it takes a long time to grow tubercle bacteria.

When a few bacteria are transferred to a test tube of sterile broth, they have at the start all the food and all the oxygen they want, and they multiply readily for a while. One hundred multiply to 200, 400, 800, 1,600 and so forth, and everything goes well. Then, a million is reached, and then 2 million, finally 4 million. And suddenly, there is no more oxygen. The 9 parts per million which were in the sterile broth, are used up, and now the only oxygen available is that from the air above. When the oxygen in the broth is exhausted, more will dissolve, but the process of dissolving is slow, and only those lucky bacteria which are on the very surface get this oxygen. These continue to multiply at the old rate, and use up all incoming oxygen so that further down, there is absolutely no oxygen, and consequently no oxidation and no combustion. The zone of oxidation is limited to a layer which reaches not further than ⅛ to $\frac{1}{16}$ of an inch below the surface.

For those bacteria which must have oxygen for respiration, life is largely limited to the surface of liquids, and many of these bacteria have developed a trick to stay on the surface. They grow in a thin film over the surface of the liquid. The star performers among them are the vinegar bacteria which develop a thick, slippery membrane on the hard cider, the so-called mother-of-vinegar. This consists entirely of the bacteria and their gelatinous membranes. The skum yeasts make a white, dry, brittle layer which is often seen on the brine of dill pickle barrels or brine pickle tanks. The molds depend so entirely upon air that they grow only far enough into a liquid to get the moisture and food, but their main growth is above the liquid, like reeds growing in a swamp.

Below this surface where bacteria, yeasts or molds absorb all oxygen, is a large store of food, available for all that can make use of it without oxygen. A large number of organ-

isms can do that, but others cannot, and they simply die because they cannot get air. A new type of tuberculin has recently been developed by asphyxiating the tubercle bacteria. This is done by placing the culture in a vacuum.

Many species of bacteria can utilize their food also in the absence of air. Digestion under these circumstances is not as complete. The yeasts of the *Saccharomyces* type can digest the sugar by changing it to alcohol and carbondioxide. They would prefer combusting it, but when there is no oxygen, they can get along with this kind of partial digestion which we call fermentation. The lactic acid bacteria change sugar to lactic acid, the colon types ferment it to a number of different substances, and produce at the same time plenty of gas which is half hydrogen and half carbondioxide. Some of the *Clostridium* species make butyric acid and gas, some can make marshgas. The marshgas of the marshes comes from cellulose which is decomposed by *Clostridium* in the mud, far away from air and oxygen.

Similarly, proteins can be decomposed without oxygen into many different compounds among which ammonia is usually conspicuous. Some species produce very bad smelling products, again especially the *Clostridium* group which brings about the stinking putrefaction.

This decomposition of organic matter without oxygen, called the anaerobic decomposition, is the consequence of the very slight solubility of oxygen in water. The bacteria have adapted themselves to these conditions in the course of a billion years, and some of them have completely forgotten how to use oxygen. They do not combust their food even when oxygen is plentiful, e.g. the lactic acid bacteria do not use oxygen.

Anaerobic conditions occur commonly in nature. Where organic matter accumulates for one reason or another, bacteria soon multiply to such numbers that all oxygen is consumed as soon as it dissolves in the liquid. When leaves sink to the bottom of a pond, when the earth becomes water-

logged by heavy rains, when a dead animal rots in the woods, or an angle-worm dies in its hole, or animal excreta cover the ground, deterioration is so rapid that not enough oxygen can diffuse in, and anaerobic conditions prevail.

It is this very ability of bacteria to decompose organic matter only partially and incompletely that makes these organisms so valuable to us. If the streptococci and the clostridia and yeasts and molds would digest their food completely to water, carbon dioxide and ammonia, this book would never have been written. It is the lactic acid of the streptococci, the butyl alcohol and acetone of the clostridia, the alcohol of the yeast, the Roquefort flavor of some molds and the citric acid from others, and the penicillin from a third group that makes these microbes valuable to us, as a small but interesting part of our civilization.

Chapter Four

THE RELATIVES OF BACTERIA

The Chapter heading may suggest to some readers that this is a chapter on protozoa, but that would be an error. Protozoa are animals, bacteria are plants, not very impressive ones, quite far removed from a rose or a cabbage, but plants nevertheless. Bacteria are the simplest and smallest of the fungi. The largest fungi are known to everybody under the name of mushrooms—or toadstools if you are a pessimist. The medium-sized fungi are the molds, which are still somewhat like plants, and the smallest fungi are the bacteria and the yeasts. The yeasts are larger than bacteria, and multiply in a different way, but they are closely associated with bacteria in bringing about fermentations and deterioration of organic matter. The molds also join these two groups in causing deterioration as far as their peculiarities permit. Molds must have very much air. They do not grow well unless they are at least partly out of water, stretching their hyphae into the air. They do not reproduce normally when completely submerged, whereas that is the ideal condition for most bacteria and yeasts.

The closest relatives of the bacteria are therefore the yeasts and the molds. Protozoa belong to an entirely different class of organisms. Their outstanding characteristic is the great diversity of forms, their endless variations of beautiful designs with a wealth of most intricate detail, their flagella and cilia, all in great contrast to the depressing monotony of form in bacteria and yeasts. The reverse is encountered in a surprizing variety of metabolic activity by bacteria and yeasts while most protozoa have the relatively simple metabolism of higher animals, oxidizing most of their food completely. On account of this latter property, protozoa occasionally cooperate with bacteria in the deterio-

ration of certain materials, notably in sewage purification, but usually, their activity is limited to devouring bacteria which are one of the main foods of protozoa.

The yeasts are called budding fungi, for they do not multiply by dividing into two equal parts like bacteria, but produce a little bud which gradually grows to full size. That makes quite a difference. If a bacterium divides in two, both halves are equally old. We could not very well call one the parent of the other. With yeast, there is no doubt that one is the mother cell, or parent cell, and the other is

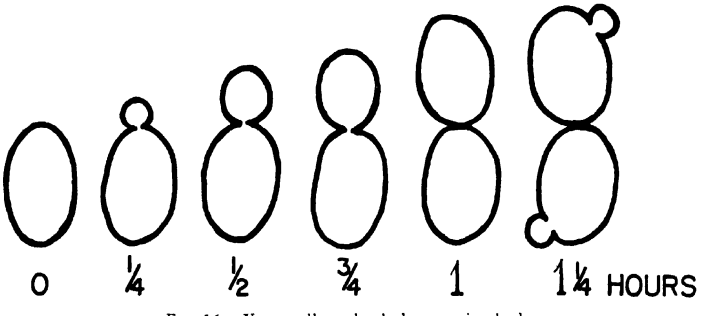


FIG. 16. Yeast cells multiply by growing buds.

the daughter cell or offspring. If the younger yeast cells remain attached to their parents as they often do, we can recognize the successive generations, as in Figure 2.

Bacteriology is not an exact science like mathematics or physics, but merely a biological science, and in biology, there is always an exception to everything. That holds true with yeasts too. One small group multiplies by fission, similar to bacteria. The first species of this group to become known was the yeast used by the Kaffers of South Africa to make some sort of beer.

The forms of yeasts vary even less than those of bacteria. The standard type of cell is ellipsoid. The ellipse may become so short as to be a circle, making a spherical cell, or very elongated, making a sausage-shaped cell. There are

Frankfurters and Bolognas and headcheese, but no fundamentally different forms of yeasts are known.

Yeasts are much larger than bacteria; that must be kept in mind for we have already seen that size is an important factor in biology. The alcohol-forming yeasts are about 5×7 microns while bacteria average 0.8×1.5 microns. A yeast cell weighs about 150 times as much as an average bacterium.

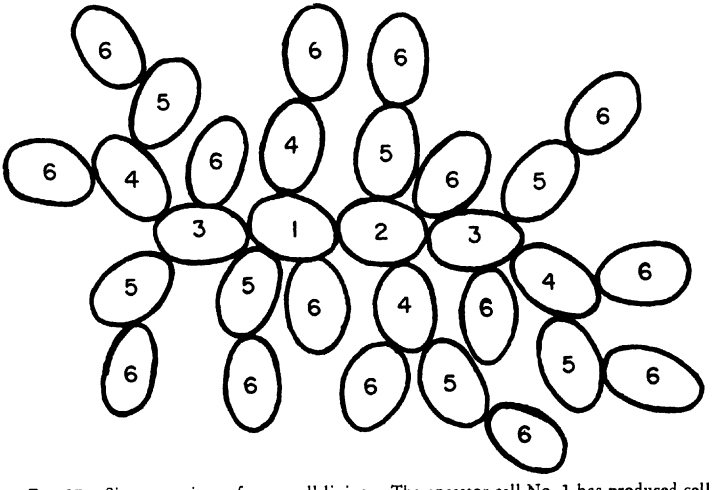


FIG. 17. Six generations of yeast, all living. The ancestor cell No. 1 has produced cell No 2, then they produced each a new cell No. 3, then the four produced each a new cell No. 4, each of the eight produced the 8 cells No. 5, and so forth.

Some yeasts produce spores. They are not very ambitious about it, and it is a very difficult task to name a newly found yeast because the specialist who divides the yeasts into sporeforming and non-sporeforming, may have to wait many weeks before he can state with any degree of certainty whether or not the new yeast forms spores. However, that is an exclusive worry of the taxonomist. Yeast spores have little practical importance as they have none of the resistant characters of the bacterial spores. They are easily killed by heat and disinfectants.

The role of the yeasts in nature is essentially the same as that of bacteria. Their main object is the deterioration of organic matter. Yeasts are more limited in the kinds of organic matter which they can attack, but that is a minor issue. Some can live only with plenty of air while others do well without oxygen.

Elaborate systems of classification and nomenclature have been established by the experts in this field, but we shall meet in this rambling discussion only three groups: *Saccharomyces*, *Mycoderma*, and *Torula*.

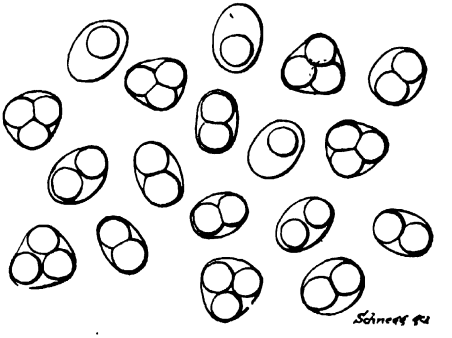


FIG. 18. Spore formation of yeast. (From H. Schnegg. Gärungsorganismen. Courtesy of the author.)

To the genus *Saccharomyces* belong the yeasts that make large amounts of alcohol. They are the sporeformers. Most of the cultivated yeasts for the manufacture of beer, bread, whiskey and industrial alcohol are very closely related and usually considered varieties of the same species, *Saccharomyces cerevisiae*. They are almost spherical, only slightly elongated, and show large vacuoles when they are full-grown. The wine yeasts are again one species with many varieties, called *Saccharomyces ellipsoideus*.

In future discussions, the term "wild yeasts" will be used occasionally. This is not a botanical term; it merely indicates unwanted types interfering with the regular fermentation. Some of them are *Saccharomyces*, some are *Mycodermas*

and *Torulas*. One of the great troubles in brewing in Hansen's time (1880) was *Saccharomyces pastorianus* which left the beer cloudy and would not settle.

Mycoderma, meaning fungus skin, is the name for yeasts that make a thin, dry, white skum on the surface of liquids. If shaken, the skum breaks, the cells separate, make the

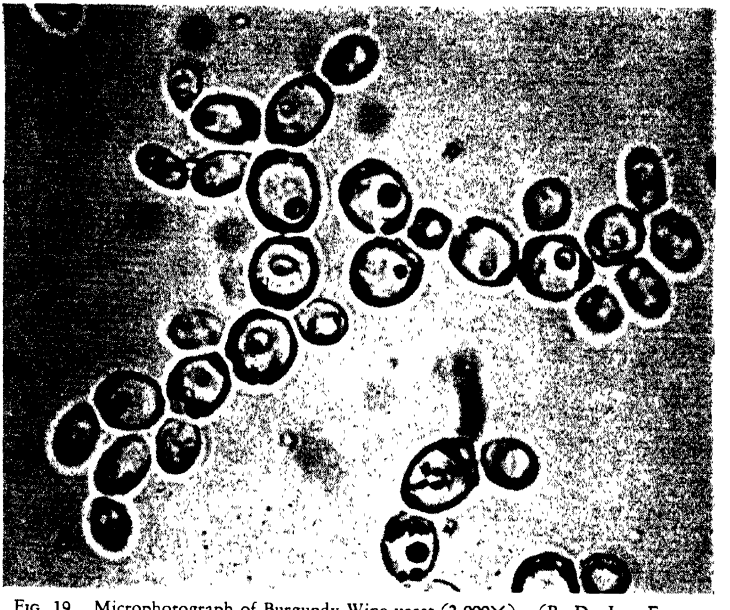


FIG. 19. Microphotograph of Burgundy Wine yeast (2,000×). (By Dr. Jean Ferguson Hofstad.)

liquid cloudy, and settle slowly to the bottom. In a short time, a new pellicle has formed on the surface. Eventually, such skum if undisturbed may become quite thick. It may be more than one inch thick on tanks of brine pickles. The Mycoderma cells sometimes look like wine yeast, but are usually more sausage-shaped.

Mycodermas make practically no alcohol; they are oxidizing organisms, and that is why they develop best on the surface. They love organic acids, especially lactic acid,

and are found wherever such acids are found, in sour milk, cheese, sauerkraut, but also in fruitjuices.

Torula is a different type again. Some Torulas are perfectly spherical, although elongated species are known too. They can produce a little alcohol, but oxidation is the preferred mode of metabolism.

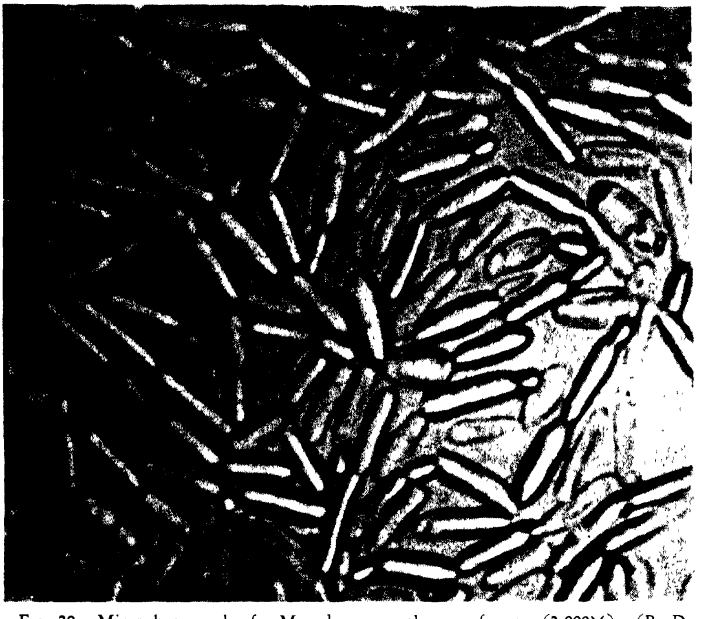


FIG. 20. Microphotograph of a Mycoderma, partly out of water (2,000X) (By Dr. Jean Ferguson Hofstad.)

The molds are in a class by themselves. There has never been any doubt that molds are plants. They look like plants. They grow from a seed, called the spore, which swells and germinates by sending out a sprout which grows into a long thread and branches again and again. In this way, a mass of threads is produced which resembles the root system of a plant. This mass of threads is called "mycelium"; the single thread is called "hypha." The entire class of molds is known to the botanist as *hyphomycetes*, or thread fungi.

That the molds belong to a higher class of fungi than bacteria and yeasts is evident by the formation of specialized fruiting organs. These so-called "sporangia" are sent

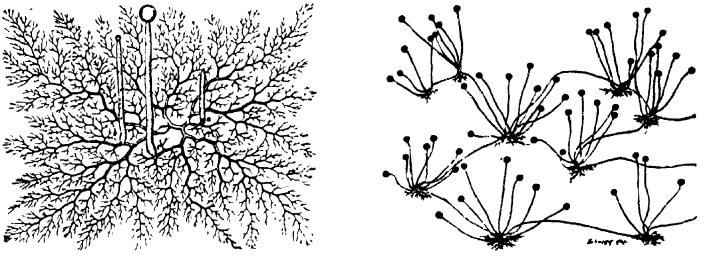


FIG 21 Complete Mold Plants. About 60× magnified. *Mucor Mucedo.* (From F. Lafar: Technische Mykologie.) *Rhizopus nigricans.* (From H. Schnegg: Gärungsorganismen.)

straight up into the air, so that they protrude over the mycelium. The mycelium is partly on the surface of the liquid or solid medium on which the mold grows, and partly

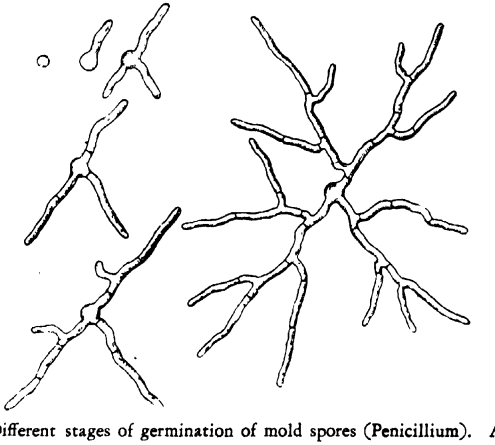


FIG. 22. Different stages of germination of mold spores (Penicillium). About 200× magnified. (From H. Schnegg: Gärungsorganismen.)

below the surface to provide water and food for the entire plant. These fruiting bodies produce the spores which are the seeds of the molds.

Since the spores are produced in the air, not under water, they are dry, and the least draught will whirl them into the air. They are too small to settle easily, and are carried by

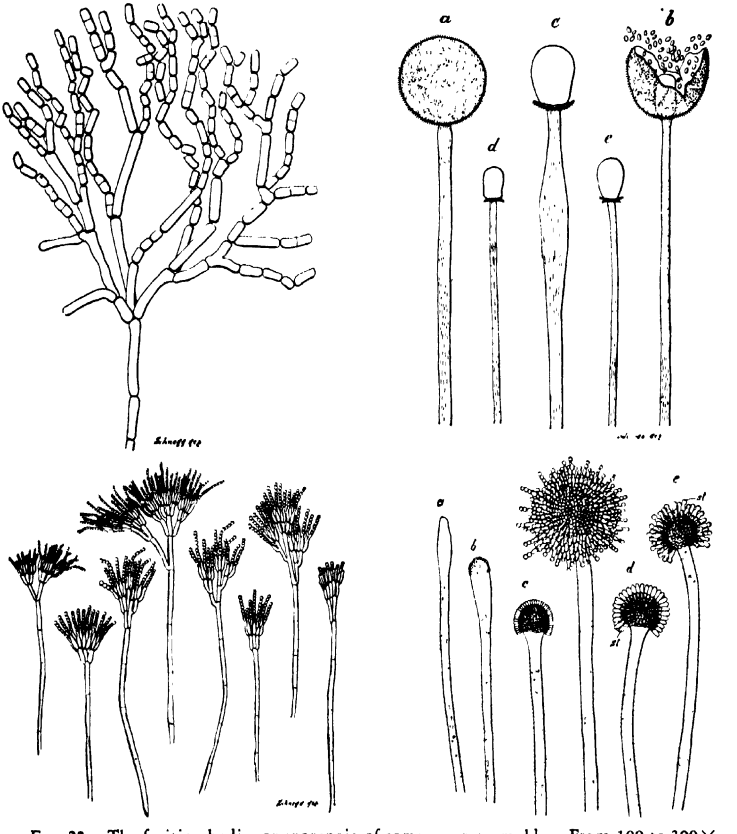


FIG. 23. The fruiting bodies or sporangia of some common molds. From 100 to 300× magnified. (From H. Schnegg: Gärungsorganismen.)

Above: *Oidium lactis* — Above: *Mucor mucedo*
Below: *Penicillium digitatum* — Below: *Aspergillus oryzae*

the wind over long distances. Bacteria always multiply in water, and are carried by air only when the water dries. Molds are designed by nature to be spread by wind like dandelion seeds, but bacteria are not.

With some molds, the spores are formed very simply by breaking the thread into a number of pieces. This is the case with *Oidium lactis*, the whitish milk mold which feeds on lactic acid like the mycodermas, and covers the surface of sour milk with a firm yellowish pellicle. It is found together with mycoderma wherever lactic acid is produced.

The common blue and green molds are usually either *Penicillium* or *Aspergillus*. They have more elaborate fruiting organs. *Penicillium* is characterized by a branched stem which is flat, while *Aspergillus* develops a spherical swelling at the end of the stem which sends out spores in all directions. The drawings illustrate the differences better than they can be described in words. Only the spores are colored blue or green, or sometimes black or yellow; the mycelium is always perfectly transparent and looks like cotton wool to the naked eye. A young mold plant which looks white today may turn green over night by making spores.

These two groups can live on a great variety of foods. Blue or green molds are seen everywhere. Rotting of apples and oranges is usually caused by *Penicillium*, grapes are frequently spoiled by the black *Aspergillus niger*. But wherever organic matter is kept damp, with plenty of air, these two types of mold are likely to make their appearance, together with many other types.

One more type of mold should be given some consideration, namely the *Mucor* group. They develop their spores inside of a spore sack which breaks when the spores are ripe. The Mucors are the largest of the molds. Their fruiting bodies can be recognized with the naked eye. Their sporangia are black or brown, and even their mycelium is sometimes brownish. Berries are often spoiled by Mucor, or by Rhizopus which belongs to the Mucor family and spreads very rapidly by runners like strawberry plants (Figure 21).

Many other types of hyphomycetes are known, with many different types of fruiting bodies, some of them very pretty. But they are too plant-like to fit well into the book

title which promised a treatise on microbes. They play a minor role in the subject matter discussed here, and they are mentioned largely because they can produce certain decompositions which are very difficult for bacteria. And then, some molds have become domesticated, notably those needed to ripen Camembert and Roquefort cheese. Their spores can be bought like we buy vegetable seeds. Some other molds are harnessed by the chemical industries. *Aspergillus niger* makes citric acid from molasses, and *Penicillium notatum* makes penicillin of recent fame in medicine.

Chapter Five

BACTERIA DIVIDE WHEN THEY MULTIPLY

That is not as contradictory as it sounds. When you divide a piece of bread, you have two pieces; you have multiplied the number of pieces by dividing. You may object that it is not a real multiplication because the total amount of bread is not increased. That is true, but that is also true with bacteria. If one bacterium divides in two halves—and all of them do—the two halves together are not bigger than the original mother cell. (As bacteria are sexless, it is becoming stylish to speak of parent cells rather than mother cells.) After the division is completed, the two young cells grow until they have about doubled their size, and then they divide again.

This kind of multiplication by division leads to some interesting results. One of them is the precise mathematical way of the increase of the bacterial population. One makes two, two make four, four make eight, the following generations have sixteen, thirty-two, sixty-four cells and so forth. If you remember your mathematics, you may realize that this is a geometrical progression beginning with $2^0 = 1$.

$$2^0 \quad 2^1 \quad 2^2 \quad 2^3 \quad 2^4 \quad 2^5 \ldots 2^n$$

The bacteriologist makes use of this geometrical progression for some of his calculations.

Another interesting feature is that when the two young cells are born, there is no mother or parent cell left. The two young cells are usually considered to be of the same age, although some doubt has been recently raised. If these two young cells grow up and divide again, four cells are born, and these are again of the same age, and no old parents are left. This continues, and even when we have millions and billions, they are all of the same age. Certainly, even

with bacteria, there are some ambitious individuals that simply cannot help multiplying faster than the rest, and some laggards which are in no hurry at all, and take their own sweet time. But there are no old parent and grandparent and great-grandparent cells mixed with the young generation. All cells in a growing culture are young and lively. That is quite different from human or animal populations.

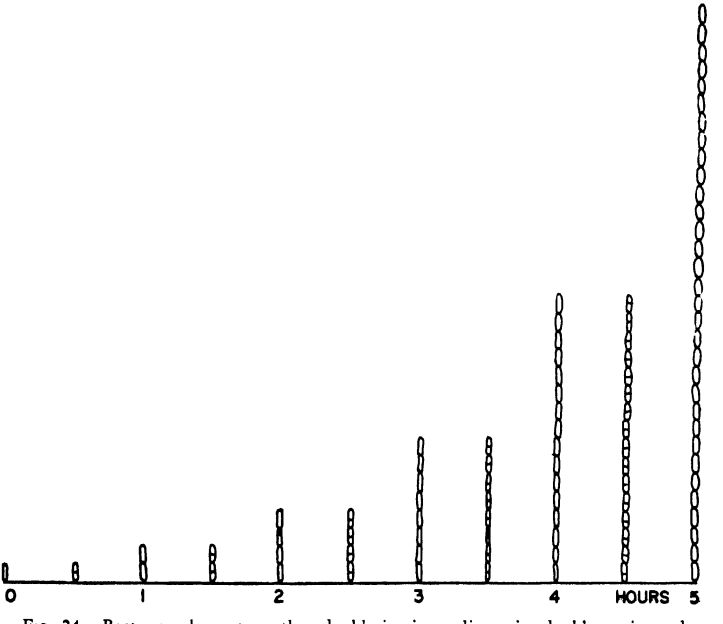


FIG. 24. Bacteria split in two, then double in size, split again, double again, and so forth, and this is the only way they multiply.

Yeasts behave somewhat differently in this respect. They are not only larger in size, but have other traits of larger organisms, and one of them is their mode of reproduction. They make a little bud which grows larger and larger until it reaches the size of the parent. Then it separates; at least it completes the cell wall and thus partitions itself off against the parent cell, but may stick to the parent just the same. Both the parent and the offspring now produce new

buds, which grow to maturity, and then all four start new buds. The mathematical aspect is really like that of bacteria: one makes two, two make four, four make eight. But biologically, they are not of the same age; we have successive generations. This is evident with those yeasts which remain attached to each other. The entire family tree is plainly recognizable, as in the Figure of Chapter Three.

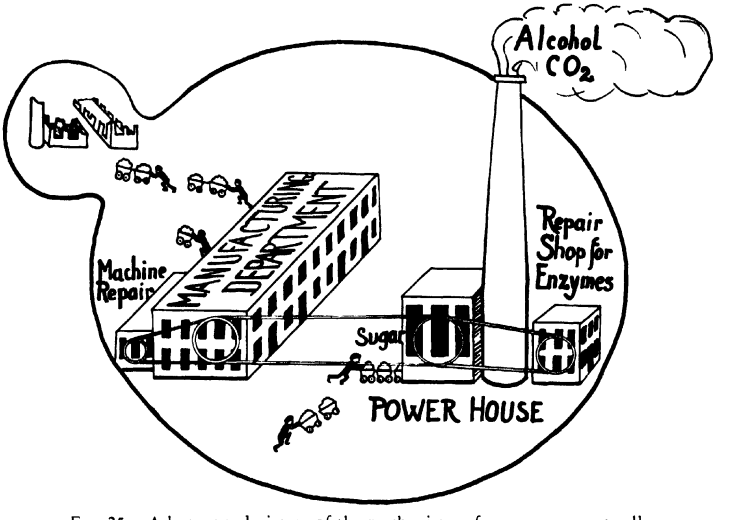


FIG. 25. A humanized picture of the mechanisms of a growing yeast cell.

When a large plant grows, that seems simple. A plant consists of many cells, and the cells divide, so that there are more cells, and these cells grow and divide again, and so the plant gets larger and larger. But bacteria and yeasts consist only of single cells, and we may wonder how a single cell grows. The cell gets food, and this food is somehow changed into cell material.

In the manufacture of bread yeast, the yeast cells are fed sugar and ammonium salts and the necessary minerals. That is enough for them to grow. But the yeast cells do not consist of sugar and ammonia; they are composed of a cell wall, of protoplasm which is protein, of enzymes which are

different proteins, of fat, and of glycogen which is a starch-like substance. All of these substances must be manufactured inside the yeast cell from the simple food it gets. That is no small task. No chemist could do it. A very complex machinery must exist inside the cell, and that is indicated in Figure 25 which cannot be called a microphotograph of a growing yeast cell, but is a translation from nature into human conceptions.

The construction of a cell is like that of a house. We need building material, workmen, and a building plan. Building materials may be very different. A house can be built of mud, wood, brick, cement, glass, or plastics. The wood may consist of trees felled by the pioneer who builds his own house in several months of hard work; or it may be entirely prefabricated, ready to be set up by two men in one day. These two men would be helpless if a door were missing. They could not make the door and thus could not finish the house. The pioneer made his own doors, windows, foundations, fireplaces, shingles, from the trees he himself had felled, and some rocks. The construction of bacteria differs in the same degree. The energy obtained from respiration or fermentation represents the workmen. The more energy, the more work can be done. The larger the prefabricated units for the cell, the quicker is growth. It takes far more time and far more energy to build a cell from carbon-dioxide and nitrate than from sugar and amino acids.

To make the phenomenon of growth clear, it is necessary to consider it from the chemical angle, and as there is no time or space here to teach the rules and regulations of chemistry, the subject must be presented in a very unorthodox way.

All things consist of molecules, even the air. Some consist of only one kind of molecules. They are pure chemicals, like salt, or sugar, or water, or silver. Others consist of several different types of molecules. Flour consists mainly of starch molecules, but also contains water molecules, and some cellulose molecules from the bran, and even a little

protein. A bacterium consists mostly of water; 75% of its weight is water. The membrane may perhaps be pure cellulose, but the inside, the cytoplasm, contains several different proteins, nucleoproteins, and usually some reserve substances such as fat, or glycogen which is similar to starch. Then, all cells contain certain minerals, the ash constituents, such as potassium and iron salts, and phosphates and sulfates. A bacterial cell is a very complex thing.

Each molecule consists of atoms. There are about 100 different types of atoms in the world, and everything on earth, or in the entire universe, consists of some combination of one or several different atoms. If a substance consists of only one kind of atoms, it is called an element. Oxygen in the air is an element; each oxygen molecule consists of two oxygen atoms. Iron consists only of iron atoms, copper only of copper atoms. The nitrogen gas of the air is also an element. It consists of two nitrogen atoms. There is a third gas in the air, carbon dioxide, and that is not an element, for it is made up of one carbon atom and two oxygen atoms. Water contains one oxygen atom and two hydrogens.

Our organic world is made up largely of four kinds of atoms; carbon (that is coal), oxygen, hydrogen, and nitrogen. Sometimes sulfur and phosphorus atoms come in, too. It is quite marvelous that all the different parts of all the different plants and animals consist merely of different combinations of these few kinds of atoms.

Two factors bring about this great variety: The *number* of atoms of each kind which is present in the substance, and the *order* in which the atoms are put together. The carbon atom is the most important and it forms the backbone of all organic molecules. Hydrogen is the smallest.

Marshgas has one carbon per molecule, 4 hydrogens, no oxygen.

Alcohol has two carbons. So has acetic acid. They differ in the number of hydrogens and oxygens.

Three carbons are found in lactic acid, and in glycerine.

Six carbons are typical for the sugars. Glucose has 6 carbons, but cane sugar and milk sugar have twice 6 carbons. They are called carbohydrates because for each carbon, there are two hydrogen atoms and one oxygen atom, just enough to make one water molecule for each carbon.

Starch is made by linking a large number of sugar molecules, perhaps 50 of them, together to one giant molecule.

Cellulose is similar to starch, but it consists of many more sugar molecules welded together.

Fat as we know it is a mixture of several different pure fats. The pure fats are large molecules and have usually between 40 and 60 carbon atoms.

All these substances named above consist only of carbon, hydrogen, and oxygen. They contain no nitrogen, sulfur or phosphorus.

The simple organic nitrogen compounds are the amino acids. The simplest is glycine or amino acetic acid which has two carbons and one nitrogen, with some hydrogen and oxygen. Most of them have more carbons, but the highest is 11. They have usually one or two nitrogen atoms, one has 3, and one has 4. About 25 amino acids are known. A few of them have also one sulfur atom.

Their importance lies in their ability to be welded together to very large molecules, and these are the proteins. Proteins consist entirely of amino acids, but they are huge complexes containing at least 200 amino acid molecules, and not of one kind only, but of 5 or 10 or 20 different kinds in different proportions linked together in a very definite and orderly sequence. The protein of each species of bacterium is different from that of all other species, or of all other organisms.

That is all the chemistry we need to know for growth.

Growth is the making of large molecules from the small ones, while digestion, fermentation and respiration are processes which break up the large molecules into smaller pieces.

In the respiration of sugar, for example, the 6-carbon molecule is broken up into 6 small carbon dioxide molecules and 6 water molecules. In alcoholic fermentation, it is split in two alcohol molecules and two carbon dioxide molecules.

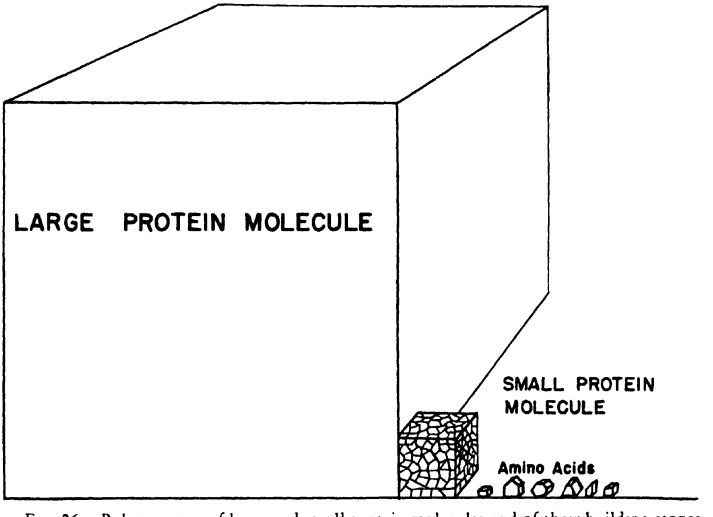


FIG. 26. Relative sizes of large and small protein molecules and of their building stones, the amino acids. (Really, protein molecules are more or less rounded, not square.)

Pepsin and trypsin digest proteins partly to single amino acids, partly to bigger aggregates of 2 or more of them.

Growth is the opposite process, the building of large molecules from small ones. The construction of a cellulose molecule from some thousand carbon dioxide molecules is the regular task of all green plants, and of the nitrate and sulfur bacteria. That is real pioneer work, starting with the simplest form of building material. It requires great skill, i.e. an elaborate growth machinery in the cell, and lots of energy. The yeasts cannot do that. They want their carbon partly prefabricated, at least in units of 3 as glycerine or

lactic acid, but they like it best in units of 6, as sugar. If they have sugar, they can use ammonia to make all the necessary amino acids for their proteins. That is no small job. It would take a chemist many a week to do that, and then, the chemist would not know how to put the amino acids together to make yeast protein.

Some of the bacteria have as much skill in building as the yeasts, or even more skill. *Bacterium coli* and *Pseudomonas fluorescens* can multiply in a solution containing minerals and ammonium salt, with no other organic matter than sugar. But most bacteria are not such experts in building. They want their nitrogen already prefabricated. They are usually able to make some of the needed amino acids, but not all. They may need only tyrosin, or cysteine, and can make all other combinations. But others have even less skill. *Streptococcus lactis*, according to the latest investigations, cannot grow unless it has the following 6 amino acids as building stones: valine, leucine, isoleucine, methionine, arginine and glutamine. If these are present in the food, the streptococcus can build all the other necessary amino acids itself, such as tryptophane, tyrosine, cystine etc. In addition to these building materials, it must have several vitamins, namely pantothenic acid, nicotinic acid, biotin and thiamin, all belonging to the B vitamins. The rest of the vitamins can be made by the cell.

With this long list of necessary compounds, it may seem surprising that it has been possible to grow these bacteria before all their requirements were known. But that is very simple. Most proteins contain all these amino acids, and the materials commonly used for the cultivation of bacteria contain all the needed vitamins. Meat extract and yeast extract are good vitamin sources, and Lister could obtain the first pure culture of *Streptococcus lactis* 70 years ago, because milk contained everything that these bacteria need for growth and multiplication, amino acids as well as vitamins.

Three things are needed to build the cell: power or energy which comes from respiration or fermentation; building material such as has just been discussed, different species having very different requirements; the third thing needed is a building plan. Molecules in a cell do not lie around helter-skelter. The more important ones must be arranged in a definite order, in spatial relation to each other. The mechanism which brings about the construction of complex molecules from simple parts must be very near the enzymes which furnish the necessary energy. The mechanism which makes the cellulose for the cell wall must be near the cell wall because the finished product, cellulose, is not soluble in water; it cannot be transported from one place to another.

Many other reasons exist for the assumption of a very definite, orderly arrangement of the important, large molecules in the cell, but we have no conception at all of this arrangement. The "humanized" picture of the yeast cell mechanisms is not based on any knowledge. It cannot possibly be correct. But it points out the mechanisms that are necessary, and depicts them in analogies. Nature's mechanisms are much simpler than man's. So little is known about the actual growth process that for many decades to come, there is not the slightest hope of making a real blueprint for the construction of a cell.

Of the greatest importance biologically is the rate of multiplication. The seventeen-year locust is 17 years old before it lays eggs, while a fruit fly is adult in 2 weeks. The legal age for marriage of girls is 14 to 16 years, the female elephant reaches maturity in about 14 years, cows have their first calf when 2 years old, guinea pigs can be bred at the age of 2 months, but though "pigs is pigs," bacteria beat all records. If all conditions are right, some species can reproduce 15 minutes after they are born, and that rate may be kept up as long as the food lasts. The consequences are amazing. We get into figures which are eclipsed only by the federal debt and by astronomical distances.

If we assume only one cell division per hour, we have after 10 hours 2^{10} bacteria which is 1024, or one thousand in round numbers. Each of these thousand, after another 10 hours, has multiplied again to a thousand which makes the total population a million, and after 30 hours, they will have increased to a billion, always provided that the food does not give out. In 40 hours, it is a trillion.

That may sound like a large number of bacteria, but it does not seem so to the bacteriologist. If a single lactic acid bacterium is put into a quart of sterilized milk, it would multiply in a warm room at the rate of about one generation per hour, and after 40 hours, the quart of milk would actually contain about a trillion bacteria. But that would be the end of their proliferation. The milk by that time has become so sour from the lactic acid produced by these bacteria that no further multiplication can take place.

However, some bacteria, e.g. the colon bacteria of our intestine or the putrefying Clostridium species, can double in 15 minutes. At that rate, one bacterium would develop to a trillion in 10 hours. This is not quite so because the very rapid multiplication is not kept up throughout the entire time, but it would take these species less than 20 hours to multiply to a trillion if all conditions are maintained favorable for reproduction.

Not all bacteria multiply so rapidly. The tubercle bacteria and their closest, harmless relatives which are commonly found in the soil, do not seem able to divide faster than once in 6 hours. We have no very good data on the nitrate bacteria, but they probably need nearly 24 hours to double. That may be because they have such a difficult task of construction. They start with carbon dioxide and nitrate, and very much chemical reduction (which requires energy) and very much organic synthesis (which requires more energy) is necessary to make a solid structure out of these small stones. Compared with this, the bacteria which

grow in broth build their cells from large, prefabricated units of amino acids and carbohydrates.

The same bacterium may multiply rapidly or slowly, depending on conditions of life. One very important factor is temperature. We are so used to comparing all life to our

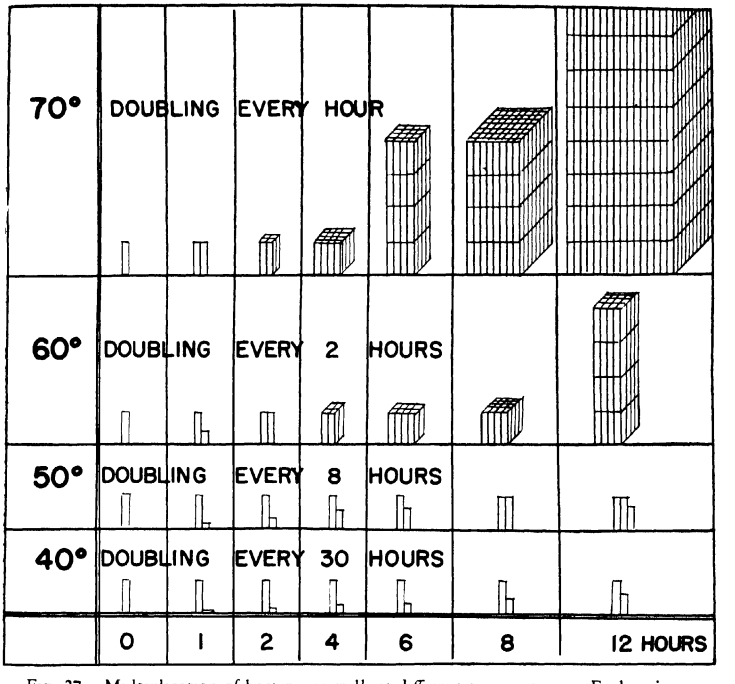


FIG. 27. Multiplication of bacteria in milk at different temperatures. Each unit represents 100 bacteria.

standards of man, mammals and birds that it is difficult to imagine organisms which have no such thing as "body temperature." If we put the bacterial culture on ice, the body temperature of all cells is 32°F., and if we put them in the incubator, it is 100°F. At 100°, the machinery of most bacteria runs rapidly, whereas at 32°, most bacteria have come to a complete rest, only a very few can multiply at a

very slow rate, dividing once in 24 to 48 hrs. The effect of different temperatures on bacterial growth is shown graphically in Figure 27.

As always in biology, there are exceptions and special adaptations. Some cold-loving bacteria do fairly well at 32°, better at 50 to 60°, and cease to grow when the thermometer rises above 70°. Others again, the heat-loving or thermophilic bacteria do their fastest growth at about 130°, some can even multiply at 160°, but when the thermometer falls to 110°, they are very slow, and below 100°, it is too cold for them to multiply. These thermophilic bacteria live not only in hot springs, but are common soil bacteria. The steaming manure piles are kept hot by their rapid respiration. They may cause the so-called self-heating of hay and straw, and unfortunately, their spores sometimes get into canned vegetables, survive the heating and spoil them if the cooling is slow and inefficient. All canned goods are now cooled in water as soon as they come out of the "retort" to prevent thermophilic spores from germinating. When the contents are cooled to 100°, they are safe; that is too low a temperature for thermophiles.

Chapter Six

SOME BACTERIA ARE TOUGH

The ancient biologists took it for granted that animals can originate spontaneously from inanimate matter under certain conditions. This belief was maintained to the middle ages, and the well-known Alchymist van Helmont (1577–1644) gave a recipe for the creation of mice. But in the 17th century, a new critical attitude based on experiments rather than on arguments began to develop, and in 1675 the Italian monk Francisco Reni proved experimentally that the maggots in putrefying meat do not originate in the meat by spontaneous generation. He kept the flies away from the meat by a fine gauze, and then, no maggots developed.

However, this did not necessarily apply to the tiny animalcules discovered by Leeuwenhoek, and the conflict of opinions about the origin of these microorganisms lasted for nearly two centuries and it was again Pasteur who finally settled it. For a long time, the two opposite views were founded on two apparently contradictory experiments. The Anglican minister John T. Needham reported in 1745 that broth boiled in hermetically sealed vessels, when kept for a few days, contained again infusoria which could have originated only by spontaneous generation. The Italian Abbot Spallanzani in 1765 found that such "infusoria" developed in boiled broth only if the outside air had free access. If the air had been heated before coming in contact with the broth, no animalcules developed. The opponents claimed that this heating had changed the air so that it was no more fit to sustain life. Thus, both parties held to their opinions.

Much later, Franz Schulze in 1836 renewed the dispute. No bacteria developed in boiled broth if the air over the broth had been bubbled through sulfuric acid. Schwann in

1837 observed the same thing if the air was passed through a heated tube before reaching the broth. Finally, Schroeder and Dusch in 1853 repeated Schulze's experiment, but substituted cotton for sulfuric acid. This cotton could not possibly cause a chemical change of the air. However, while these experiments gave conclusive results with some liquids such as boiled vegetable infusions or clear broth, the method did not work with milk. In boiled milk, also in meat, small organisms developed sometimes after boiling, no matter how

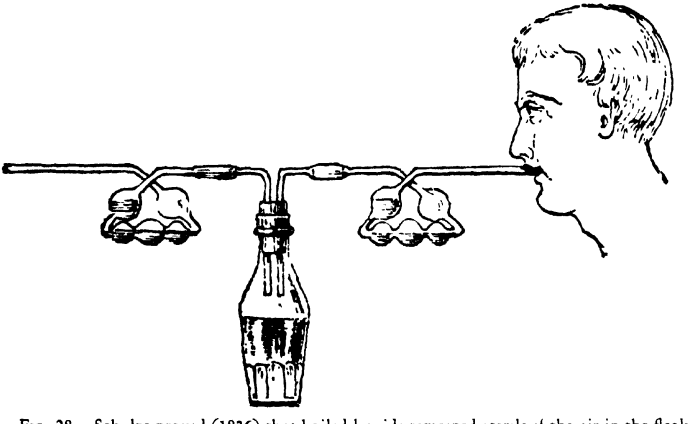


FIG. 28. Schulze proved (1836) that boiled liquids remained sterile if the air in the flask was renewed by passing it through sulfuric acid.

carefully the air which came in contact with these decoctions had been filtered through cotton.

Finally, in 1860, the Paris Academy of Sciences offered a price for the best experiments to clarify this problem of spontaneous generation. The price was given to Pasteur who proved that a sufficiently long heating will make any substance sterile, i.e. unable to produce microorganisms. He heated his liquids in flasks with long drawn-out swan necks which were later known as Pasteur flasks. When the flasks cooled after boiling, the air entered through the long neck, but so slowly that all microorganisms, being heavier than air, settled out in the neck. He showed that the liquid

was still fit for fermentation by transferring a tiny amount of fermenting material into the liquid which resulted in multiplication of the microorganisms and beginning of fermentation.

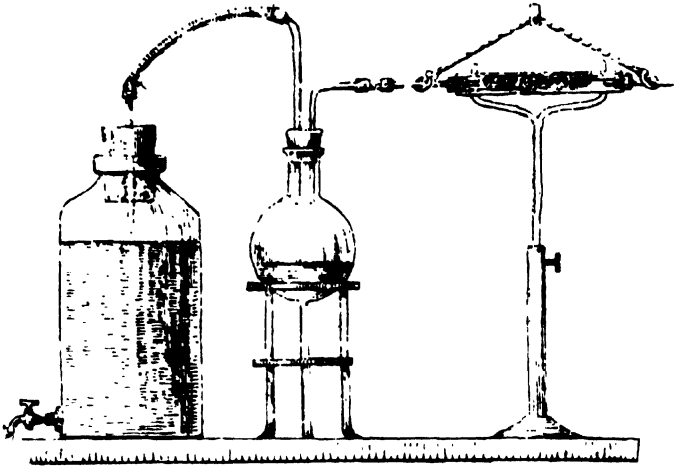


FIG. 29. Schroeder and Dusch proved (1853) that boiled liquids remained sterile if the air in the flask was renewed by passing it through cotton.

Thus it was proved conclusively that all living things, even the bacteria, originate only from their ancestors; that there is no spontaneous generation; and that the old sentence —*omne vivum ex vivo*—could not be questioned. This did not

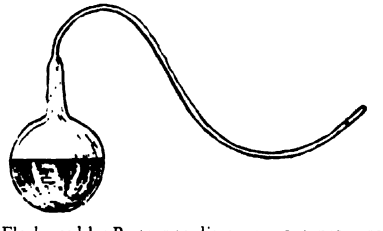


FIG. 30. Flask used by Pasteur to disprove spontaneous generation.

explain, of course, how the first bacterium had originated. However, since this is entirely a matter of speculation, practically identical with the question of the origin of all

life on earth or in the universe, the explanation must be left either to the Bible which makes a statement without proof of eye witnesses, trusting to its own authority, or to the textbooks on evolution which offer theories based on analogies, but also without the final experimental proof.

There was a short revival of this dispute over spontaneous generation ten years later which led to an important dis-

FIG. 31. Pasteur flasks still in use in a modern brewing laboratory. (Courtesy of Piel Brothers, Brooklyn, N.Y.)

covery. In 1872 the English scientist Bastian questioned Pasteur's evidence by showing that a turnip decoction to which cheese had been added and in which all acid had been neutralized, could be boiled for several hours in a Pasteur flask, and after a few days' standing would always contain innumerable bacteria. Duclaux, the student and biographer of Pasteur, and later his successor, stated that "Pasteur beat about the bush for a long time, and during this time, his ideas were rather confused."

Just at this time, the German botanist Ferdinant Cohn had undertaken a classification of bacteria. He repeated and confirmed Bastian's experiments and finally revealed that some bacteria are able to form spores which can tolerate very high temperatures, especially when they are enclosed in cheese particles. This discovery explained all the difficulties which Schulze, Schroeder and Dusch, and Pasteur had experienced when trying to sterilize milk or meat through boiling. It explains also the difficulties which we have to this day in the preservation of meat and vegetables by heat.

The formation of spores has been discussed in the first chapter. As has been shown there, most bacteria cannot produce spores. The non-sporulating bacteria are easily killed by heat. The Pasteurization of milk is usually accomplished by holding the milk for half an hour at 145°F., and this kills 99% of all bacteria, and 100% of the disease bacteria. Some species require higher temperatures to be exterminated. This is especially true of the thermophilic species, mentioned in a previous chapter, which are regularly found in soil and frequently in milk. Even the most outstanding thermophilic bacteria, however, cannot survive boiling while the spores of most bacilli do survive this ordeal for some time.

The reason for the great resistance of bacterial spores to heat is not definitely established. A number of theories have been proposed, but none is generally accepted. No other living organism can tolerate such high temperatures. While the theory is lacking, the facts are well known. They have been carefully studied because they are of the greatest practical importance. In the laboratory of the National Canners Association, Bigelow measured the heat resistance of some spores which had spoiled canned vegetables. The spores were put in vegetable juice in small glass tubes which then were sealed airtight. The tubes were dropped in a kettle with oil heated to a constant temperature. After a certain heating time, one tube was taken

out and cooled, 5 minutes later another, and so forth. This was done at many different temperatures. All tubes were placed in an incubator and if any spores had survived the heating, they would germinate and make the vegetable juice cloudy. Thus it was easy to see how much heat the spores could tolerate.

Bigelow tested 15 different bacteria from spoiled canned vegetables in this way and found that they varied in their heat resistance, but not very greatly. The observed killing times were as follows:

Heated to		Range of killing times of the different species	Average killing time
212°F.	100°C.	788 -834 miuntes	811 minutes
221	105	383 -405 "	394 "
230	110	117 -122 "	120 "
239	115	40 - 44 "	42 "
248	120	11 - 12 "	11 5 "
257	125	3.9- 4.6 "	4.25 "
266	130	1.7- 2.2 "	1 95 "
275	135	42 - 54 seconds	48 seconds
284	140	36 - 54 "	45 "

These and similar investigations were the basis on which modern canning factories have based the heating times for the preservation of vegetables. Fortunately for us, very few of the pathogenic bacteria are capable of forming spores, and it is therefore relatively easy to exterminate them.

Dry spores can withstand much higher temperatures than wet spores. The sterilization of glassware, cotton, instruments and other dry materials for bacteriological or medical purposes requires heating to 340°F. for at least one hour. Liquids in which bacteria are to be grown, usually designated as culture media, are heated to 250°F. for 20 to 30 minutes. As this is higher than the boiling point of water, this is done under pressure in an autoclave. This is the same

apparatus which the housewife calls pressure cooker, whereas in the canning factory, it is called retort.

Before autoclaves were manufactured on a large scale, laboratories and housewives used to preserve their products sometimes by discontinuous sterilization. This was a clever trick to fool the spores. The material was first boiled to kill all non-sporulating bacteria. The spores survived, and after the liquid had cooled off, the spores would begin to germinate and produce vegetative cells. These cells then multiplied as all bacteria do by doubling and redoubling. No new spores would be formed for some time because spore formation is a sign of old age and does not occur as long as the bacteria have plenty of good food. Thus, all spores were changed into vegetative cells, and if such a liquid was heated again on the following day, these cells would be killed, and the liquid would thus have become free from spores. This method takes a good deal of time, but gives good results with most materials if all rules are observed. The main rule is that after the first heating, the material must be kept at a fairly warm temperature to insure the germination of all spores and it must be reheated before any new spores can be formed. The customary method is to heat on three successive days to catch the last of the spores. However, this procedure does not always produce sterility, because some spores have a very delayed germination, especially old spores which have been dry for some time. It is impossible to sterilize soil, for soil experiments, in this way, and milk frequently spoils after even four successive days of heating.

There are other means of sterilization besides heat. We have hundreds of different disinfectants and antiseptics. Most of them cannot be removed from the sterilized materials, and thus will preserve them permanently. This is desirable in some cases, but as most disinfectants are also quite toxic to man and animals, their use in food preservation is extremely limited. The law allows only very few exceptions.

Drying will kill a large percentage of bacteria, but hardly

ever all of them. In soil dried for 60 days, 64% of the bacteria died. If this soil is wetted, the surviving bacteria will double in one hour, and after two to three hours, the old number is reached again. This simple calculation shows that drying has not affected the bacterial population of the soil very much.

FIG. 32. In a shoe store, shoes which have been tr.ed on by a customer can be sterilized with ultraviolet light to prevent infection of the next customer with such diseases as athlete's foot. (From Science Illustrated of September 1944.)

The same is true with dried fruits. Dried raisins or prunes soaked in water will start fermenting merrily in one or two days at warm room temperature because so many yeast cells on the surface of the fruits survived drying. In the manufacture of milk powder, the milk is first pasteurized to make sure that the pathogenic bacteria in it are destroyed. Drying alone would not accomplish that, nor could the heating of the dry milk powder be relied upon.

Freezing is equally unreliable. It is practically impossible to kill all bacteria in a liquid by freezing. The number of survivors is always quite considerable although many are torn by the formation of ice crystals inside of the cells, or

FIG. 33. Meat is contaminated with bacteria only on the outside, and can be kept longer when irradiated with ultraviolet lamps. (From Science Illustrated of September 1944.)

killed through some more complex processes. For this reason, ice from contaminated water is still capable of causing typhoid fever.

A very good means of sterilization is ultraviolet light. It kills spores and dry bacteria very efficiently. Its greatest disadvantage is its poor penetrating power. Even glass will

prevent its effect almost completely, and clear water absorbs it to quite an extent. It is used in a few instances to sterilize small water supplies, but sterilization of milk or even of fruit juices is practically impossible. It has been found very useful especially in the sterilization of the air in hospitals, operating rooms and food industries.

One would think that X-rays would have the desired penetrating power. The trouble is that they penetrate too well. Radiation can cause a chemical or physical effect only when it is absorbed. Ultraviolet is often absorbed before it gets to the bacteria. X-rays not only get to the bacteria, but go right through them without being absorbed, and therefore do not harm them. If we recall how easily X-rays penetrate the many inches of living tissue in our chest when an X-ray photograph is taken, we realize how little chance there is of their being absorbed by a bacterial cell. If they are absorbed, however, which happens occasionally, they kill the cell. But they also kill the cells of human tissue if absorbed. It is not possible, therefore, to cure infections with X-rays.

Chapter Seven

CONTAMINATION

Contamination is something artificial, an invention by man. Nature knows of no contamination because Nature does not work with pure cultures. Similarly, Natuer does not know weeds because it does not grow plants in fields or rows. Nature gives chances only to the fittest. What is fittest in one place is not so fit for another place. But our earth has so very many different places with different soils and different climates, and that is why we have so many different species of plants and animals. None of them is raised in pure culture.

All the many yeasts, molds, and bacteria which we keep with great care and effort as pure cultures in the laboratory, perform, somewhere on this earth quite naturally and in competition with other microbes, year after year the same kind of chemical breakdown of organic matter that we try to bring about with pure cultures. Science and industry could not get along without pure cultures because if other bacteria were admitted, they might overgrow the culture and change the type of fermentation products which were being produced. But in Nature, they continue to exist, and somewhere on this globe, ideal conditions must exist for even the rarest type of microorganism. As long as these ideal conditions exist, this species thrives and multiplies, not in pure culture, but without being crowded out. After some time, however, when conditions are altered or the food is exhausted, multiplication of this one species ceases. Other species come to the front while the first crop of cells becomes old. Many die, some usually become dry and dormant, or form spores, ready to come back to active life and multiplication when conditions become ideal again.

We can safely say that practically all kinds of micro-

organisms can be found everywhere, blown there with the dust, carried there by rain, or on the feet of insects, on the hair of mice or the clothes of man. Of course, this will depend somewhat upon the size of the sample. One cubic centimeter looks to an average bacterium as large as 30 acres of land, 3 feet deep, would appear to a wheat seed. We have many different kinds of bacteria on the leaves of plants (about a million for every gram); we have many different species on the skin of man and animals; and about a hundred different species in every cubic foot of fertile soil. Many of them did not grow where they are found. We observe frequently bacteria in places where they could not possibly have developed, e.g. intestinal bacteria in fresh water, bacteria of the pear fire blight on a man's hand, tubercle bacteria on a green leaf. But they must have grown somewhere. Somewhere in this world are conditions, perhaps only temporarily, where the rarest species can multiply and meet the competition of all other microorganisms.

Some of these conditions are well known. Fruit juices always undergo alcoholic fermentation because yeasts are better adapted to the very acid and sweet juices than all other microorganisms. When the sugar is gone, vinegar fermentation usually follows because few other organisms can multiply in the alcoholic liquid. Raw milk will always become sour, by lactic acid bacteria, and most always, the acid will be destroyed by Oidium molds or Mycoderma, unless man interferes. Moist grains will always become moldy whereas the same grain completely submerged under water will undergo butyric acid fermentation.

We speak of these processes as "normal" or natural types of decomposition. As long as the science of bacteriology was not developed, i.e. until about 1880, it was almost impossible to enforce any type of decomposition except the normal. Wine could be made easily because fruit juices always contain plenty of yeast, and change naturally into wine. It was only necessary to pour the wine off the lees

when the yeast had settled, and to keep the air out ever afterwards so that the wine did not turn into vinegar. It was easy to make sour milk, and it was possible, with great care, to make cheese. It was easy to make sour-dough bread because the formation of acid and gas is the normal process observed when flour is wetted and stands in a warm place.

But sour-dough bread did not appeal to the spoiled American palate, and we use yeast now to leaven the dough. This is not the normal type of decomposition. Yeast does not normally grow on grains. It can be transplanted into the dough and it can multiply there slowly, but after 12 to 24 hours, the bacteria of the flour will have increased sufficiently to compete with the yeast. The baker enforces the "unnatural" yeast fermentation of the dough by using very large quantities of yeast so that the fermentation is over in a few hours. Then, the baking process ends all competition by killing friend and foe.

Before 1880, brewing must have been a very difficult art because the normal decomposition of grain mashes and malt extracts is a lactic acid fermentation. A keg of malt extract left to its natural microbial flora would never turn into beer as easily as a keg of fruit juice will turn into wine. It would get very sour, and would contain very little alcohol. The brewer must use two tricks to overcome this threat. First he adds hops which contains an efficient antiseptic, the bitter hop oil, which inhibits many bacteria, but does not hurt the yeast. And secondly, from time immemorial, the brewer sterilizes his malt extract by boiling. To this sterile wort, he adds his special yeast in large quantities. Now-a-days, it is a pure culture yeast, but 80 years ago, it could not possibly have been pure, and the art of conducting the fermentation so as to obtain a high quality product is greatly to be admired.

As soon as it became general knowledge that fermentation was caused by microorganisms, the need for pure cultures was as obvious in the fermentation industries as it was for

the scientific study of bacteria. Pasteur wrote a book about beer and its troubles, and about wine and its troubles, but he had no means of obtaining pure cultures. It simply was not possible at that time, since no method had been developed. While Pasteur unquestionably helped the beverage industries greatly, and taught them methods to cure and avoid abnormal fermentations, he could not get at the root of all troubles because he had no pure cultures.

As early as 1878, Joseph Lister in England thought of a clever method to obtain pure cultures. He was then interested in the souring of milk. By means of an ingenious little syringe which delivered as little as $\frac{1}{100}$ of a drop, he spread a measured amount of sour milk on a glass plate, let it dry and counted the bacteria of this little drop under the microscope. He found that one drop contained about 100 million bacteria. Then he diluted this milk a million times with sterilized water and with his special syringe, put $\frac{1}{100}$ of a drop of this dilution into 5 glasses of sterilized milk. This should have put just one bacterium into each glass, but bacteria can not be distributed quite so evenly. Four of his glasses remained sterile, but in the fifth, the milk turned sour and coagulated. That was the first pure culture obtained from a single cell and present-day bacteriology still recognizes the *Bacterium lactis* of Lister as a pure species, although the name has been changed to *Streptococcus lactis.*

By this method, we can get only the kind of bacteria that is most numerous. The other species could not be isolated until Robert Koch in 1881 developed the gelatin plate method. He dissolved gelatin in broth, put it in testtubes and sterilized it. This nutrient gelatin would solidify into a firm jelly at room temperature. To isolate a pure culture from any material, he mixed a very small amount of this material in a tube with melted nutrient gelatin and poured this gelatin onto a sterilized glass plate which was cooled by ice. The gelatin solidified, and the bacteria distributed in it could not move any more. They had ample food all

around them, and began to multiply, and in a few days, each single cell had grown to such large numbers that they formed a whitish speck in the brownish-yellow gelatin (see figure 40). The gelatin plate had been kept covered by a sterilized glass jar, so that no dust could fall onto the plate. Each of the whitish specks must have come therefore from a bacterium in the material under test, and was very probably a pure culture, coming from a single cell. All that remained to

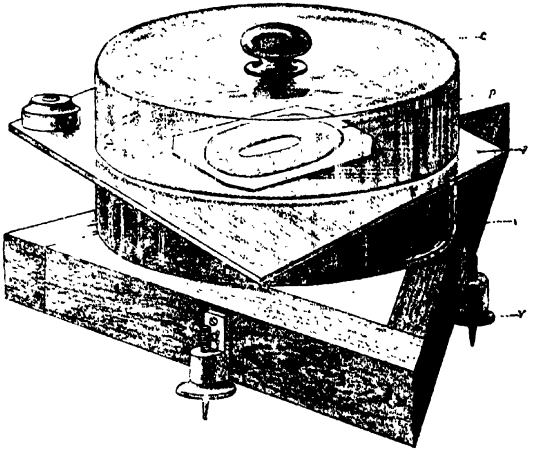


FIG. 34. The equipment used by Robert Koch (1881) to obtain pure cultures. The nutrient gelatin was poured on a sterile glass plate cooled by ice underneath, kept horizontal by a leveling device, and covered with a glass jar. (From Cornil Babes: Les Bactéries, 1890.)

be done was to transfer a little of the white speck, called a colony, with a flamed needle into a tube of sterile broth, and a pure culture was obtained.

This method had one great disadvantage. Gelatin melts so readily that the plates could not be kept at body temperature. Further, many bacteria digest gelatin rapidly, and thereby make it liquid. One of Koch's assistants, Dr. Hesse, had great trouble with these liquefying bacteria, until his wife who cooked all his culture media for him, made a helpful suggestion. Mrs. Fanny Eilshemius Hesse

had been born and educated in the United States, and was familiar with the jellying power of agar which melts only near the boiling point. One day, she substituted agar for gelatin, and it was a perfect success, so perfect that it has remained the standard jellying agent to this day.

The inconvenient plain glass plates gave way to round glass dishes with fairly tight-fitting glass covers, called Petri dishes after their inventor. Only such terms as "pouring plates" or "plate count" remind us now of the original

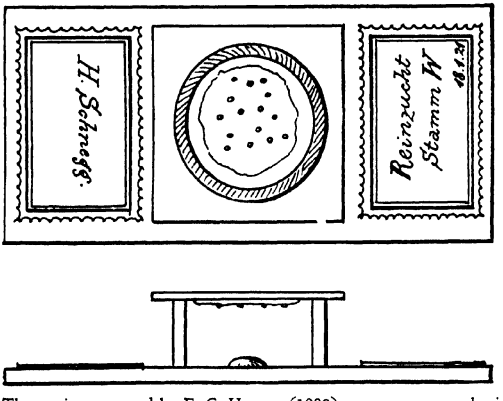


FIG. 35. The equipment used by E. C. Hansen (1880) to grow yeast colonies from single cells on a coverglass in a small moist chamber. (From H. Schnegg: Gärungsorganismen.)

equipment by which Koch succeeded in isolating the first pure cultures of the tubercle bacteria. But essentially, we still use the plating method as the simplest means to get pure cultures.

Credit must also be given to Emil Christian Hansen of the Carlsberg Laboratory in Copenhagen who used gelatin in a similar way a year before Koch's publication. Hansen wanted reliable pure cultures of beer yeasts. He mixed a tiny bit of his yeast with sterilized beer wort (i.e. malt extract) to which gelatin had been added. He spread this on a coverglass, and mounted it on a glass ring over a glass slide so that an air space remained between gelatin and glass.

This gelatin layer was then inspected through the microscope. Any yeast cell that was found to be separated from all others, was marked by a drop of ink on the outside of the coverglass, and if it grew during the next days to a small colony, it was sure to be a pure culture.

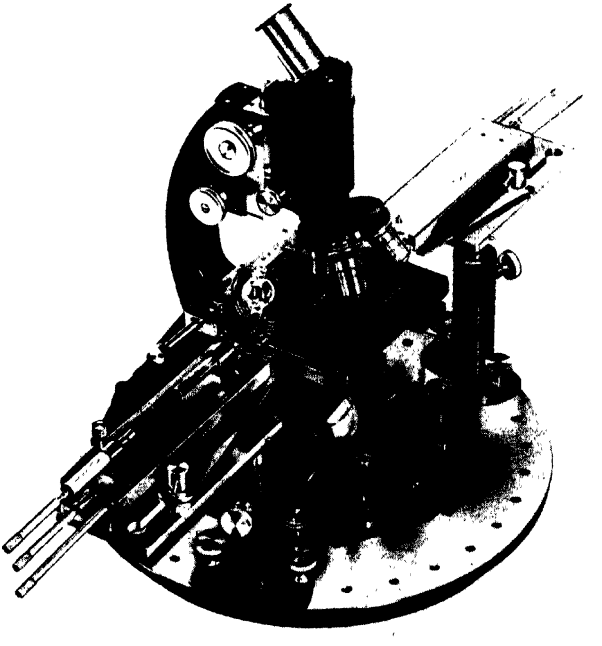


FIG. 36. A micromanipulator for the isolation of single bacteria cells from a liquid. (Courtesy of Bausch & Lomb Optical Company, Rochester, N.Y.)

As bacteriology advanced, the requirements for absolute purity of bacterial cultures became more and more rigid. For certain studies, it became necessary to be absolutely certain that the culture was obtained from one single cell and not from two cells adhering to each other, even if they were sister cells. This could be assured only if the single

bacterium to be transferred was selected under the microscope. Several types of elaborate equipment have been constructed by which it is possible to draw a single bacterium into a microscopic glass tube, and to deposit it into a drop of broth from which it can be transferred to a larger tube. This is called the Single Cell Technique, and the instrument is the Micro-Manipulator.

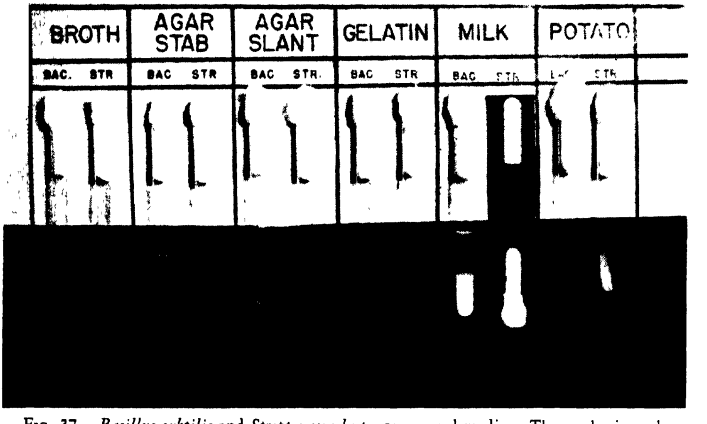


FIG. 37. *Bacillus subtilis* and *Streptococcus lactis* on several media. The air-loving, skum-forming, gelatin- and milk-digesting bacillus is contrasted with the air-avoiding, not protein-digesting, milk-curdling streptococcus.

Once a pure culture is obtained, it is easy to keep it pure. As a rule, such cultures are kept in testtubes with nutrient broth or agar or milk or cider, depending upon the preferences of the bacterium under consideration. The tubes are closed with a cotton plug which filters out all bacteria from the air, but permits the filtered air to come to the bacteria. If we are dealing with anaerobic bacteria, which do not like air, some melted paraffin is poured onto the broth to seal the culture airtight.

Since bacteria cannot with certainty be recognized by their microscopic appearance alone, it is important to determine the changes they may produce with sugar or protein or other foods. They are grown in meat gelatin to see if they

can digest it. If they do the gelatin becomes liquid. They are grown on sugar solutions to see if they make acid; this is tested by an indicator. They are grown in milk to see if the milk becomes acid, or alkaline, or coagulated, or whether the casein of the milk is digested. They are grown on slices of potato to test their effect on starch, or on a pure sugar-mineral solution to see if they can utilize the nitrogen of the

FIG 38. Tools of the bacteriologist. At left, various filters, in the background Petri dishes sterilized in the metal can, test tubes, platinum needles and loops. At the right, several media and dilution bottles with sterile water, and fermentation tubes; in the foreground pipettes.

air. Special media are employed to find out other special properties. Gas formation is measured in special fermentation tubes, and the gas thus obtained is analyzed to find out whether it is carbon dioxide or hydrogen or marsh gas. Hydrogen sulfide is produced by some bacteria in sufficient amounts to be smelled. Other agreeable or disagreeable odors may help to identify certain species. For each group of microorganisms, special culture media have been designed, and the manufacture of bacteriological culture media and of the materials needed for this purpose has developed into a little industry separate from the chemical industries.

Quite an important technique in the work with pure cultures, mixed cultures and contaminations, is the counting of bacteria. It is often necessary to know how many bacteria are present, and what kinds. The quality of a water supply is judged by the total number of bacteria present, and especially by the number of fecal bacteria which indicate sewage contamination of the source of supply. Many different methods have been worked out for special cases. Bacteria are just a little too small to be counted easily under the microscope, but the microscopic counting of yeasts offers

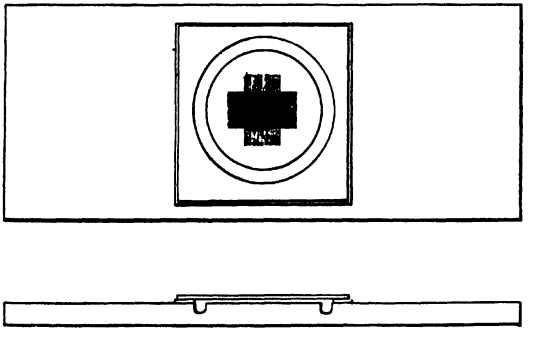


FIG. 39. Counting chamber for counting yeast cells in a liquid. Top view and cross section. (From H. Schnegg: Gärungsorganismen.)

no difficulty. A drop of the beer or the bread yeast suspension is put into a counting chamber of glass with a special ruling. The same equipment is used in hospital laboratories to count the red and white corpuscles in blood. The average number of yeast-cells per square is determined by counting 50 or 100 fields. That can be done with a magnification of 100 to 300 diameters which does not strain the eye. The average number per field multiplied with 4,000,000 gives the number of yeast cells per cubic centimeter.

This method does not apply to bacteria because they are too small. Higher magnifications are necessary, and for these, the regular counting chamber cannot be used. Special glass chambers have been constructed for the counting of

bacteria, but they are not very accurate, and are not used much.

The preferred method is the "plate count." A measured quantity of the liquid to be tested is mixed with nutrient agar and poured into a Petri dish. After 1 to 7 days, the colonies which developed on the agar are counted. Presumably, each colony came from one cell. If one cubic centimeter of water gave 57 colonies, we conclude that the

$\frac{1}{1000}$ g of soil $\frac{1}{10,000}$ g of soil $\frac{1}{100,000}$ g of soil

FIG. 40. The common method of counting bacteria in soil. Each white speck is a colony of bacteria grown from a single bacterium in the soil. The plate to the right, made with 1/100,000 gram of soil, has 72 colonies, therefore, the soil contained 7,200,000 bacteria per gram.

water was quite good because it contained only 57 bacteria per cc. When a liquid contains many bacteria, it is greatly diluted before mixing with agar. If a bacteriologist counts the bacteria in a sample of soil he weighs out one gram and puts it into a bottle with 99 cubic centimeters of sterilized water. He shakes it for a long time, and with a sterile pipette, puts $\frac{1}{10}$ of 1 cubic centimeter into a sterile Petri dish. This contains the bacteria of $\frac{1}{1000}$ gram of the soil. Then, he transfers from this bottle 1 cc. of the liquid into another bottle with 99 cc. of sterile water. The water becomes only slightly cloudy. He shakes for several minutes, and then

removes 1 cc. of this liquid and puts it into a Petri dish. Into a third Petri dish, he puts $\frac{1}{10}$ cc. These dishes have thus received the bacteria from 1/10,000 and 1/100,000 gram of the original soil. Then, melted nutrient agar, cooled to 110°F. is poured into the three dishes, and is swirled around rapidly to mix with the soil bacteria before it begins to jell. Each bacterium will then grow where it is fixed and produce a

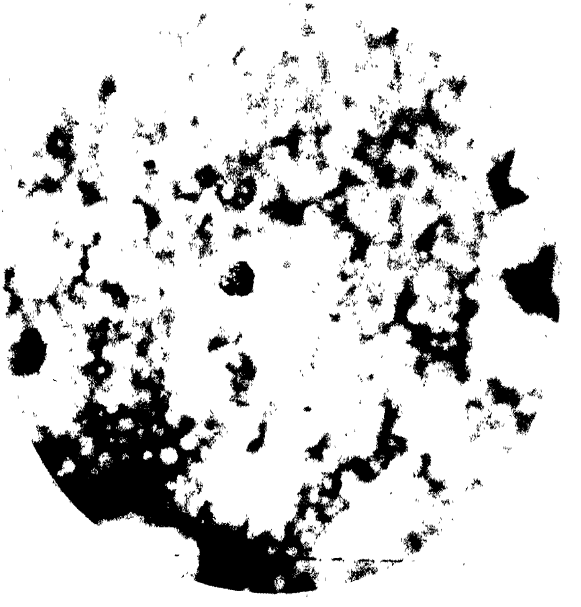


FIG. 41. Microscopic appearance of high-grade milk, 600X magnified. Not a single bacterium is visible, only one cell from the cow's udder. The mottled background is due to milk fat. (Courtesy of Agricultural Experiment Station, Geneva, N.Y.)

colony which can be seen with the naked eye. Figure shows the colonies which grew in these three soil dilutions in 48 hours.

This method is very good, but we must have patience because it takes 2 or more days before all bacteria have grown to colonies which can be readily seen. The dairy bacteriologist cannot wait that long when he tests the milk of

each patron. He works somewhat like Lister did. He measures with a delicate pipette $\frac{1}{100}$ cc. of milk, and spreads this on a glass slide so that it covers exactly one square centimeter. He dries and heats this milk film so that it sticks to the glass, then he puts it into a solution of methylene blue which dyes the bacteria deep blue. He washes off

FIG. 42. Microscopic appearance of milk held at room temperature for 16 hours, beginning to sour, 600X magnified. The inset shows Streptococcus lactis, which causes the souring, 2000X magnified. (Courtesy of Agricultural Experiment Station, Geneva, N.Y.)

the excess dye, dries the glass slide and counts, under the microscope, the number of bacteria in each visible field. The average number multiplied by a certain factor tells him exactly the "germ count" of the milk.

This chapter cannot be closed without mentioning one still simpler method for roughly estimating the bacteria in milk, namely the Reductase Test. You put a specially pre-

pared tablet of a dye, methylene blue, into a measured amount of milk and shake it so that the milk gets deep blue. Then you put the tube with the blue milk in water of 70°F. and see how long it takes before the milk turns white. The bacteria in the milk need oxygen for respiration. There is very little oxygen in the milk, and when that is used up, the bacteria take oxygen from the blue dye. Thereby the dye loses its color and the milk turns white. If the milk has a million bacteria, the oxygen is used up in 10 minutes. With only a thousand bacteria, it takes many hours before the milk turns white.

Chapter Eight

BACTERIA AMONG THEMSELVES

The preceding chapters must have given the impression that at times, bacteria are rather crowded. A billion bacteria in a cubic centimeter of sour milk, a few million in a cubic centimeter of sewage, ten million in a gram of soil, that suggests really close quarters, and one may wonder how bacteria get along when rubbing elbows not only with their own kin, but with many other different kinds of bacteria as well.

Rarely do we find more than a billion bacteria per cc., and if these are uniformly distributed, they are ten microns apart from each other. That is only $\frac{1}{100}$ of a millimeter, and may seem to us a very short distance indeed, but to bacteria, that is not so small since it is fully ten times their diameter. Let us compare that with people in a city. The average width of a person may be estimated as one foot. If men were as crowded as bacteria in a full-grown culture, every person would be 10 feet apart from another person at the right, at the left, at the front, at the back, above and below. That is not very different from life in a one-room apartment in a large apartment house. Many families have much closer living quarters, and in an office skyscraper or in a department store, people in daytime are much more crowded than a billion bacteria in one cubic centimeter, without suffering seriously.

This comparison is not quite correct, however. People in a city should not be compared with bacteria in a liquid, but with bacteria in a colony on agar. The people of a city obtain their food from the country far away, and so do bacteria in a colony. In a liquid, a bacterium feeds on the food which is available between itself and its neighbors. That is not very much, considering the enormous appetites

of bacteria which easily consume their own weight of food in one hour and continue to do so every hour of the day or night until all food is gone.

We might also compare a bacterial culture consisting of many million cells with a large plant or animal consisting of many million cells. There, the cells are absolutely as close as possible, touching each other. The food problem for the individual cells of a large plant or animal can be solved only by an elaborate system of circulation of a fluid which carries all the necessary food to the individual cells and takes away their waste products. Although animal cells have not nearly the voracity of bacteria, there are so many of them to be fed, that the blood circulates more than 100 times in an hour, carrying food from the intestine and oxygen from the lungs to each cell, and taking away carbon dioxide to be released in the lungs, and other waste products to be discharged by the kidneys. A bacterial colony also consists of millions of cells touching each other, but the system of circulation of food is lacking, and hence, a colony soon stops growing.

Here is the first problem in bacterial association, namely the competition for food. With pure cultures in the laboratory, competition does not result in fight. As bacteria have no mouths and must let their food diffuse into them through the cell membrane, this diffusion will be about equal for all cells, and as far as we are able to tell, no individual can get more food by greater skill or sly tricks. All cells, after having digested their food, give off the fermentation products in the same way, by letting them diffuse from the inside to the outside. This also is regulated by physical laws without favors to anyone. When the food is exhausted or when certain products of fermentation, such as alcohol, acid or ammonia, become too concentrated, fermentation ceases, the cells become old, and finally they die.

When several kinds of bacteria are present, which is the common status in nature, different relations are possible.

The various species may take no notice of each other. In soil, we find cellulose-destroying bacteria, starch digesters, nitrogen-fixing bacteria of the Azotobacter and the Clostridium type, nitrite and nitrate bacteria, and so forth. Organisms living on different types of food do not compete with each other, except for oxygen if that becomes scarce in a very wet soil. It may happen, of course, that one species produces an excessive amount of acid or ammonia so as to affect the acidity of the soil, which would cause a great change in the bacterial population, but that is a rare occurrence. Nitrate bacteria do not compete with cellulose bacteria, nor with sulfur bacteria, nor with starch bacteria. But there are several species of cellulose bacteria, and they compete with each other. So do the different sulfur bacteria and the different nitrate bacteria. Usually, for each type of food, a number of different species is present in the soil. If cellulose is attacked by a rapidly growing and a slowly growing species, the rapidly growing kind will outgrow the slower species, and will get most of the cellulose. When the cellulose supply is used up, more of the rapid species will survive.

In soil, the "soil climate" composed of temperature, moisture content, aeration, soil acidity and other factors, is frequently more favorable for one species than for another, and that decides the rate of multiplication for each type. Since food in the soil is rather scarce, it rarely happens that the fermentation products of one species suppress other species. This is more common in the decomposition of dead plants or animals. Another selective agency is present in soil, namely the protozoa. These are found in fairly large numbers, several thousand per gram of soil, and as they live almost exclusively on bacteria, they may prefer certain species to other species, and thus keep one kind in check.

In freshly pressed grape juice, many different kinds of wild yeasts are present and start to grow and multiply for a while. But the alcohol produced by some of the fermenting

yeasts becomes stronger and stronger, and one species after another of the wild yeasts is overcome by the alcohol and drops out of the race. Finally the one type of wine yeast which can tolerate the most alcohol is found in an almost pure culture.

In raw milk, the situation is similar. All the many bacteria from the straw of the bedding, from the hairs and the udder of the cow, and from the pail and the hands of the milker, find plenty of food in milk and begin to multiply in it. Among the many species are some streptococci which ferment the milk sugar to lactic acid. This acid disturbs most of the others, they lag behind and soon quit multiplying altogether, and finally, only one or two species of streptococci hold the field almost entirely to themselves.

Wherever food is plentiful, dominance of one type of bacteria is the rule, but none of these dominant cultures holds the field permanently. The hierarchy of the wine yeast in grape juice is followed by a hierarchy of vinegar bacteria which suppress the yeast completely. The dynasty of the streptococci in milk gives way to the acid-destroying *Oidium lactis*, and when all acid is gone, anarchism rules and many different bacteria which are no longer held back by acid, cause a putrefaction of the milk proteins, eliminating the Oidium as well as the streptococci.

But not all life is fight. We find excellent examples of cooperation among bacteria. Vinegar bacteria do not grow well in grape juice. They like alcohol best, and only after the yeast has fermented the grape juice do the vinegar bacteria really thrive; but the yeast does not profit by this process. Similarly, *Oidium lactis* wants lactic acid for best development, and is not happy in fresh milk. Thus, one type of microorganism prepares the food for the other. In the case of Oidium and streptococci, the relation is for a while a real symbiosis, of benefit to both parties. The streptococci make acid so rapidly that they suffer themselves from too much acid, and begin to die. When *Oidium*

decreases the acid, the bacteria can ferment again, and they remain alive much longer in association with *Oidium* than in pure culture.

It has been mentioned before how the nitrate bacteria cannot feed on ammonia, but must wait for the nitrite bacteria to change the ammonia to nitrite. The molds which ripen Camembert and Rocquefort cheese cannot thrive unless some bacteria make the fresh cheese acid. Azotobacter cannot digest cellulose, but if cellulose-splitting bacteria are present, Azotobacter develops well by getting its food from cellulose indirectly. Starch is indigestible for all yeasts, but they can ferment it when certain molds capable of changing the starch to sugar grow in the same medium. Such relationship is sometimes called commensalism, or eating together at the same table.

All this competition is rather good-natured, on the basis of live and let live. But a few bacteria and molds have developed a vicious means of keeping other bacteria away. They secrete strongly poisonous substances which paralyze other bacteria and prevent them from multiplying. A few such cases were known 60 years ago, especially the pus-forming *Pseudomonas pyocyanea* which kills many other species. Such antagonisms are often observed when agar plates of a mixture of bacteria are kept for some time. Around certain colonies, a free zone is observed in which no other species can develop. While this was known to most bacteriologists, it did not occur to any of them to try to obtain these toxic products in pure condition to cure infections in man and animals.

This was first suggested by Dubos who found some strongly "antibiotic" substances produced by a soil bacillus. From the culture, a substance called Gramicidin was isolated which showed good promise for the treatment of pneumonia and streptococcus infections. Then, other bacteriologists remembered that a similar substance had been described some time ago as a product of a mold, *Penicillium notatum*. This

penicillin proved even more efficient than gramicidin and, what is very important, less toxic to man and animals. Since then, many other fighting substances have been remembered or newly discovered. They are produced from quite

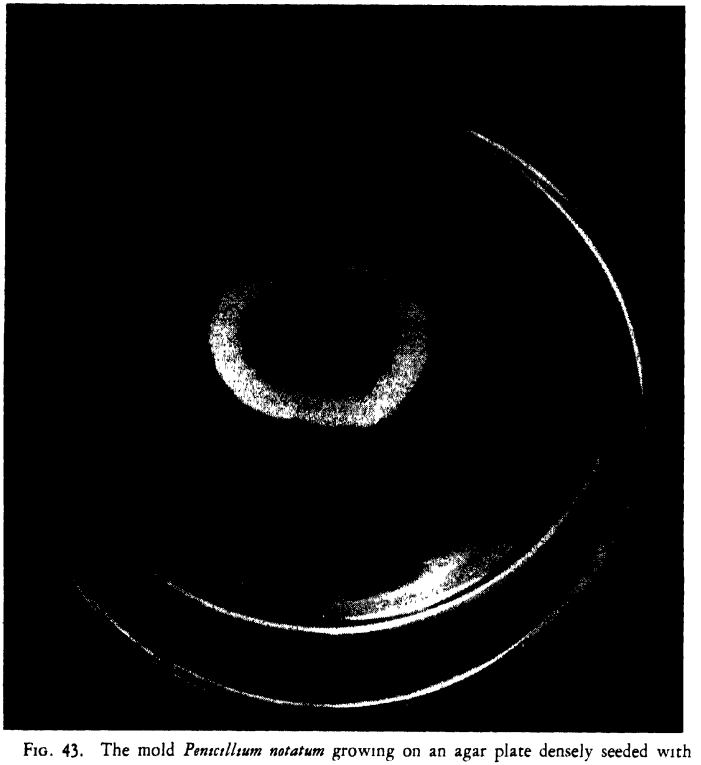


FIG. 43. The mold *Penicillium notatum* growing on an agar plate densely seeded with bacteria. The bacteria grew so thick that they made a gray haze at some distance from the mold, but could not develop near it because of the excreted penicillin. (Courtesy Cheplin Biological Laboratories, Syracuse, N.Y.)

different groups of organisms: gliotoxin by the mold *Gliocladium*, actinomycin by *Actinomyces antibioticus*, streptothricin by *Actinomyces lavendulae*, pyocyanin by *Pseudomonas pyocyanea*, gramicidin and tyrocidin by *Bacillus brevis*, and so forth.

After many tests, medical science has given preference to penicillin. The special Penicillium is now grown in large quantities in many factories in this country and in England. Its cultivation is not as easy as that of bacteria. Molds grow only on surfaces, and the provision of so much surface is a most difficult problem. It is interesting that the industry had called for help from the commercial mushroom

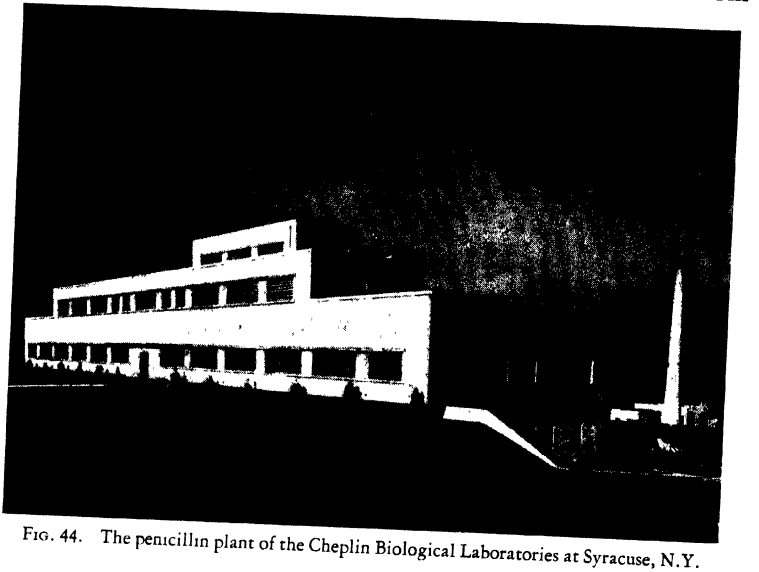


FIG. 44. The penicillin plant of the Cheplin Biological Laboratories at Syracuse, N.Y.

growers. Mushrooms are quite close relatives of the molds, and they too grow only on the surface of solid media, and require much air. In fact, the mushroom spawn is started as a pure culture on agar where the mushroom spores germinate and produce a mycelium very similar to that of molds. But now strains have been developed which will grow in the liquid if it is well aerated.

These antibiotic substances may be considered a means of defense or of aggression. They serve both purposes. Such chemical mechanisms are not at all unusual in biology; they are quite common in higher plants and animals. The ink

of the squid and the scent of the skunk are peculiar developments of chemical substances for defense. To this category belong also the bitter and toxic compounds produced by some plants, and the bactericidal property of blood, serum, milk and saliva. The rattlesnake venom and the poison of spiders must be considered primarily as aggressive mechanisms brought about by the production of peculiar chemical substances. The toxic substances formed by microorganisms are merely defensive weapons, the microbes do not feed on the dead competitor. Really, quite often the competitors are not killed, merely paralyzed, and may recover when they are put into another medium free from toxins.

Chapter Nine

FROM DUST TO DUST

"In the sweat of thy face shalt thou eat bread till thou return unto the ground; for out of it wast thou taken; for dust thou art, and unto dust shalt thou return."

This homely conception of our origin and destiny is as true now as it was when it was written by Moses or whoever else composed the book of Genesis. The line of thought underlying this unadorned expression was probably that man lives from plants which grow out of the dust, and therefore must be made from dust. After death, the body deteriorates and finally disappears completely, being gradually and inexorably changed to dust. The quotation might even imply the observation that plants grow more prolifically where people or animals have been buried. Certainly, the statement suggests a cycle, or rotation, alternating from dust to man and back to dust, or from dust to food (bread) to man to dust. This cycle cannot be completed without bacteria. They are exclusively responsible for one part of the cycle, namely for bringing man and all other living beings back to dust.

The ascendent part of the cycle, from dust to man, is well known to the biologist. All living matter is produced by growth. Annual plants grow from tiny seeds to maturity in a few months; the California redwoods continue to grow for several thousand years. Animals grow from egg cells. Maturity is reached by some insects in a few weeks, by some vertebrate animals only after many years. Animals may remain alive in the adult stage for a long time after maturity is reached, but finally, they die. All plants also must finally die, even the redwoods.

The total growth on earth in one year can be estimated. If the grass on a meadow would not decompose after it is dead and beaten down to the ground in winter by rain and

snow, the growth of one year would cover the ground about half an inch high. In 100 years, the dead grass would have piled up higher than the highest blade of grass standing up. In the forest, if the dead leaves and branches falling down every year did not decay, they would accumulate in 500 years higher than the tops of the trees. If manure did not rot and disappear, it would pile up so fast in a cattle ranch that in 200 years, it would be higher than the heads of the cattle.

But the layer of dead grass on the prairies has remained nearly the same for centuries. There is no increasing accumulation of dead leaves in the forests, or of barnyard manure on the ranches. No accumulation of dead plants or animals prevents or interferes with the growth of new plants and animals. Each passing generation makes room for a new generation by simply disappearing from the face of the earth.

This disappearance is almost entirely due to bacteria and their allies, the yeasts and molds. If all bacteria on grass, leaves, wood, or dead animals could be killed, these materials would not decompose. Wooden structures keep for many centuries if kept dry which prevents the action of bacteria. Egyptian mummies have been preserved by disinfectants and drying for over 2000 years. The remains of living things decompose readily only when bacteria have free play under proper conditions.

Chemically speaking, an organism consists of very many different substances. There is not only fat, protein and carbohydrate in the plants and animals, but the cellulose in the woody parts of the plants, the keratin in hair and horn of the animals, the chitin skeleton of insects, the lignin, tannin and resin of trees, the organic acids of fruits and vegetables, and many other substances. Every substance produced by living organisms must be decomposed by microorganisms. If there were any compound produced by a plant or animal which could not be decomposed, it would

have accumulated in the many million years and would be present on earth in enormous quantities.

This has actually happened once. The coal we burn is the product of thousands of years of forest growth which through some peculiar geological event became sterilized and escaped bacterial decomposition. Our peat bogs are in a similar condition at the present time. The organic matter of the sphagnum moss undergoes an extremely slow decomposition, especially when it is completely submerged under water. This is also true of the humus of the soil. It is not absolutely stable and decreases when it is not replenished by barnyard manure or other organic matter, but the rate of decomposition is very slow.

It must not be imagined that every bacterium decomposes all organic matter produced by living things. Bacteria specialize more or less in their food habits just as animals specialize. Lions eat only animals, cows eat only plants, pigs eat both. The anteater lives exclusively on ants, the clothes moth only on wool, and many caterpillars will eat the leaves of only one plant and refuse all others. We have seen that the appetites of bacteria are just as different, and only a few groups are really omnivorous. Most bacteria specialize on one kind of food or another.

The decomposition of a dead plant or animal is therefore not a simple process, but represents the combined work of many different types of bacteria, and they have to work for a long time before all organic matter is mineralized, i.e. decomposed to the final stage, carbon dioxide, water and ammonia. Let us consider the fate of an apple falling from the tree. It is bruised by the fall, and the organisms from the soil can get into the torn cells. As the apple juice is acid, bacteria cannot grow very well in it, but this is ideal food for molds and yeasts. The molds can force their way from one cell to the other, using the sugar for their respiration and building their new mold cells from the apple pro-

tein. In a few days, they have grown through the entire apple and destroyed its structure.

In the meantime, the yeasts dominate in the bruised parts, fermenting the sugar to alcohol. Almost at once, vinegar bacteria begin to develop, carried to the apple on the feet of fruit flies or wasps which are attracted by the smell of alcohol. The vinegar bacteria oxidize the alcohol to acetic acid, and when food becomes scarce, they oxidize the acetic acid completely to carbon dioxide and water. Thus the sugar of the apple has become completely mineralized, and its protein has been largely used to make new cells of molds, yeasts and bacteria. The acids of the apple, mostly malic acid, are completely mineralized by the respiration of the mold. The cellulose has remained unchanged so far. When the structure of the apple collapses from the destruction by the mold, it lies on the surface of the soil, and a number of different soil bacteria can oxidize the cellulose slowly to carbon dioxide and water. Or the cellulose may be washed by rain into lower strata of the soil where air has no access, and there, it will be fermented by a Clostridium to butyric acid. The butyric acid in turn cannot be changed until it gets in contact with air again when it will be oxidized competely by a new group of bacteria.

The fate of other plant materials is similar. With leaves that are not acid, there is usually no alcoholic fermentation. As a rule, an acid fermentation of the sugar and starch by streptococci, lactobacilli, or gas-forming bacteria of the colon group goes on, and the acid is later mineralized completely by still other species of soil bacteria.

Quite different is the mineralization of dead animals. The true inside of animals is usually free from bacteria. This does not refer to the intestinal canal which is really only a tube going through the body, and which must be considered as a special part of the outside. Thus, a dead mouse contains practically no bacteria in its muscles, blood or liver, but many bacteria are on its skin, and enormous

numbers are in its intestine. The skin cannot be decomposed as long as it is dry, but the intestine is moist and the tissues of the intestinal wall are readily attacked as soon as the animal is dead. There is no oxygen in the intestine, and only anaerobic bacteria can develop. Among the most prominent of these are several species of Clostridium which multiply rapidly and digest proteins easily. In this digestion process, such gases as are hydrogen and carbon dioxide are formed, and also some offensively smelling compounds like hydrogen sulfide and skatol.

This putrefaction progresses from the inside to the outside until the skin is reached in some place and the pressure of the putrefactive gases can force a hole. Thereby, liquid oozes out, the skin becomes wet, and from now on, oxidative putrefaction from the outside and anaerobic putrefaction from the inside bring about a rapid deterioration of the proteins. Frequently, flies lay their eggs on dead animals and the maggots bore holes through the skin, thereby carrying bacteria into the inside and hurrying the decomposition process, and also helping it along directly by eating some of the flesh.

The final decomposition of the proteins is again accomplished in several steps: some species breaking down the protein to peptones, others splitting the peptones to amino acids, still others decomposing the amino acids to ammonia and carbon dioxide, with some groups specializing on the decomposition of amines. The rest of the protein molecule, as far as it has not served as building material for the multiplying bacteria, is oxidized slowly to carbon dioxide and water.

The skin and hair as well as horn, hoofs, claws, fish scales etc. are not so easily decomposed. They consist of keratin, and only a few species can attack this utterly insoluble substance. Its decomposition is very slow. Other groups dissolve slowly the chitin of the insects. The bones are largely mineral matter, and bacteria attack only the

organic substances such as gelatin contained in the bones. The acidity of the soil may ultimately, in the course of years, decompose the bones chemically.

By these and similar steps, dead plants and animals become in the course of time completely mineralized. They all return to dust. It seems quite evident from this discussion that only the bacteria and their close allies, the yeasts and molds, prevent the accumulation of dead animals and plants on the surface of the earth to such an extent that they would interfere with new growth.

The limited space on the earth's surface is not the only reason why bacteria are indispensible to life. More important is their role in providing plant food. Plants are the most important organisms on earth because they can build up their entire structure from the carbon dioxide gas of the air and from water and minerals and nitrates in the soil. They obtain the enormous energy necessary for this construction from the sun light. The chlorophyl of the green leaves is the transformer of light energy into energy for growth. Animals have no such transformer. They can obtain energy only by digesting plants, or by eating other animals which grew by digesting plants. Therefore, animals, including man, are not necessary for the maintenance of life on earth, but plants are. And so are bacteria.

Plants can use nothing else but carbon dioxide, water, minerals and nitrates for growth. Air contains only 0.03% of carbon dioxide gas, but this small amount is continuously replenished by the respiration of plants and animals and bacteria, and by the decomposition of all dead organisms.

It has been shown before that bacteria will mineralize dead organic matter completely to carbon dioxide, water and ammonia. By the respiration of animals, the organic matter of their food is also oxidized so that all carbon becomes carbon dioxide. Thus, the carbon dioxide molecule which was released yesterday by putrefaction from a dead animal may today be assimilated into starch by a plant,

perhaps a lettuce plant which is eaten the next day by a man, and is released again a few hours later in the carbon dioxide coming from his lungs. It is then assimilated again by a plant which is eaten by a cow, and our carbon goes into milk fat which is used to nourish a child, and is deposited in this child's body fat. There it may stay until the child dies as an old man but finally, after death, goes into circulation

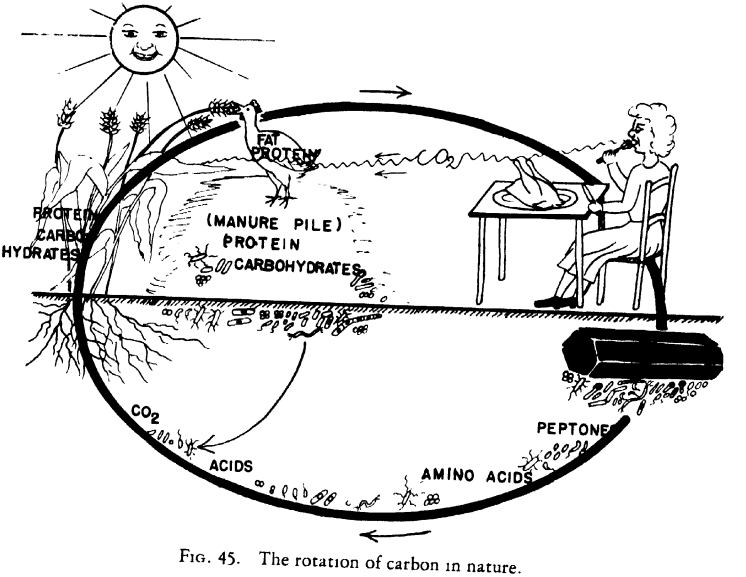


FIG. 45. The rotation of carbon in nature.

again, being released either by cremation or putrefaction. We must realize that for hundreds of millions of years, the carbon dioxide has been circulating in such cycles between animals, plants, and bacteria.

The other building materials for plants, namely the nitrates, minerals and water, are in the soil and are taken up by the plant roots. Water comes from the rain, the minerals constitute the main part of the surface of the earth and of the soil, and while they are not always easily soluble and available, the plants usually find enough in almost any soil to continue growth because they need so little of it. For the

production of large farm crops, the farmer usually adds the most important minerals, phosphates and potassium salt, and eventually lime stone, either as chemical fertilizer or in form of manure.

The most important fertilizer, however, is "nitrogen." Really, nitrogen is a gas, and four-fifths of our atmosphere is nitrogen gas. But that is of no use to the plants. It is in a form in which they cannot assimilate it, and when the farmer speaks of nitrogen fertilizer, he means nitrate or

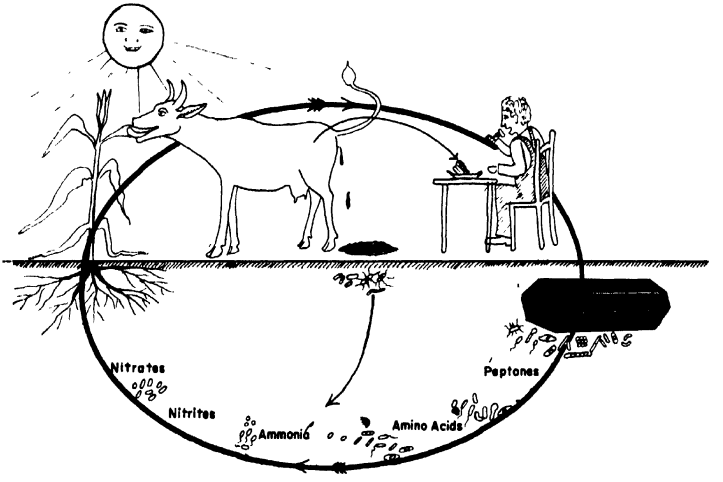


FIG. 46. The rotation of nitrogen in nature.

ammonia. Those two substances contain nitrogen in a form which the plants can take up readily, and the plants need this nitrogen to build their protein. All protein contains nitrogen, and as protein is the most important part of any living cell, nitrogenous plant food is the most important part of the soil, as far as life on earth is concerned.

Usually, soil contains very little nitrate, and it may not contain any. It is not a mineral like potassium salts or phosphates or limestone. It comes only from the decomposition of dead plants and animals or of animal excreta. The dead organisms and their excreta contain no nitrates.

Their nitrogen content consists mostly of proteins or similar nitrogenous compounds. Bacteria will mineralize all such organic matter, by cooperative decomposition, until all the nitrogen is finally changed to ammonia.

While all plants can use carbon dioxide, not all plants can use ammonia. Some of them can use nitrogen only in the form of nitrate, and practically all plants prefer nitrate to ammonia. The ammonia which is produced by putrefaction from dead organisms or animal excreta is changed further to nitrates by the cooperation of two very unusual groups of bacteria. The first group consists of *Nitrosomonas* and *Nitrosococcus*. These two organisms feed on ammonia. Ammonium salts are to them what sugar and fat and proteins are to us. They oxidize ammonia to nitrite; this is a respiration process which yields the energy for growth. They build their cells only from carbon dioxide, like green plants. They need no organic food like the other bacteria; on the contrary, sugar and proteins are poison to them. The nitrite which they make from ammonia is rather toxic to plants, but it does not accumulate in the soil because another group of bacteria, the *Nitrobacter* group, is also present, and for them, nitrite is an ideal food, in fact the only food. They oxidize the nitrite to nitrate, and thereby obtain their energy for growth. These bacteria cannot use ammonia; they must have nitrite to live. On the other hand, the *Nitrosomonas* cannot oxidize ammonia further than nitrite; it cannot make nitrate. This relationship of the two nitrate bacteria where one absolutely depends upon the products produced by the other, is one of the outstanding examples of division of labor in biology.

When the nitrate is taken up by the roots of a plant to be again built into a protein molecule, the cycle of nitrogen is completed. It is more complicated than that of the carbon, because animals cannot mineralize the protein completely. The digested protein leaves the animal body as urea, and a large group of bacteria can ferment this urea to ammonia.

Besides carbon dioxide and nitrate, the plants need other compounds, usually termed the minerals. They form the ash which remains when we burn a plant or cremate an animal. Most of these minerals retain their mineral, inorganic character even in the cell. They are mostly salts dissolved in the cell juices, and may enter a very loose combination with protein, but they are immediately released and restored to their mineral state when the protein is decomposed. This, for instance, is true for the potassium salts which the cells need very much. We can hardly speak of a cycle in such a case. Other minerals are used structurally, like the calcium in the bones, but they remain really mineral throughout their role.

This is not quite true, however, of two important elements, phosphorus and sulfur. These substances do combine with the organic matter of the cell and many proteins contain phosphorus and sulfur. Phosphorus is also an indispensable part of lecithin which is a very small, but very important part of all living cells. The organic phosphorus is easily changed by bacteria to phosphate which is an inorganic salt, and this phosphate can be used by all plants. Thus, here again, the changes are so simple that we could hardly call it a cycle.

More complex is the rotation of sulfur. In putrefaction, the bad-smelling hydrogen sulfide gas is produced by bacteria from proteins. If this did accumulate in the air for some million years, it would poison all life. This is prevented by a large group of bacteria usually called the *Thiobacteria* which feed on hydrogen sulfide. As we oxidize sugar, so they oxidize the hydrogen sulfide, changing it first to sulfur which is deposited in the cells as we might store fat from a diet of excessive sugar. The sulfur granules can be seen readily in the bacteria. When the supply of hydrogen sulfide is exhausted, the bacteria continue to live on their reserve, the stored sulfur granules, which are gradually oxidized to sulfates. Sulfate is the mineral form in which the plant

usually gets its sulfur supply. Thus, with the formation of sulfates, the cycle of sulfur is completed.

This rotation of the elements which form the organic world does not follow the same pattern for all elements. The carbon cycle is quite different from that of nitrogen, and the sulfur cycle is quite unlike either of them. Parts of these

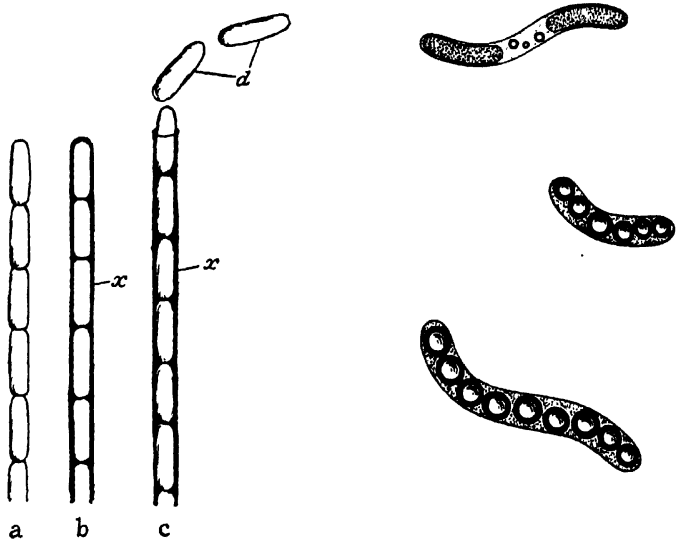


FIG. 47. Sulfur bacteria. (From D. Ellis Sulphur Bacteria Courtesy of Longmans, Green & Co.)
Left: *Thiothrix* producing a sheath from which the cells escape when mature.
Right: *Thiospirillum* with sulfur granules which serve as reserve substance.

cycles are brought about by the same bacteria, but for the final part of each element, highly specialized bacteria are necessary.

A very instructive experiment, the Micro-Universe, finds its simple explanation by the rotation of elements. The micro-universe consists of a few leaves of some tiny water plant, preferably the duckweed (*Lemna*) and a few copepods (*Cyclops*) or other very tiny water animals in perhaps two ounces of river or lake water, sealed airtight in a four ounce

chemists' flask by fusing the long neck completely shut. Life in this flask will continue for many months if the balance between animals and plants is just right, and if the flask is placed on a north window where it gets plenty of light, but no direct sun which would make the universe too hot for life.

The copepods feed on the dead leaves of the duckweed, or on the bacteria living from the decaying duckweed. The respiration of the animals and the bacteria gives carbon dioxide which is used by the duckweed to make new leaves,

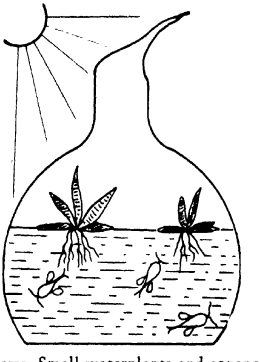


FIG. 48. The Micro-Universe. Small waterplants and copepods living for many months in a completely sealed flask.

with the help of light. This completes the carbon cycle as explained in the preceding pages, and the nitrogen cycle also follows the prescribed pattern. The plant protein is converted into animal protein. The excreta and eventual dead animals are changed to ammonia and nitrate by the bacteria of the river water, and the duckweed uses the nitrate for new growth. There is even an oxygen cycle in the micro-universe. The growing plant resorbs carbon dioxide and retains the carbon, but gives off the oxygen. The animals use the oxygen for respiration and give off carbon dioxide. There must also be a sulfur cycle and a phosphorus cycle because plants, animals and bacteria all need these elements. To provide all bacteria necessary for the various

cycles, it is advisable to put a small teaspoonful of good soil into the universe before sealing. Life finally ceases probably because something gets out of balance, perhaps because some rare element is not changed back to assimilable form.

It may cause some readers a peculiar feeling to realize that the carbon, nitrogen and sulfur atoms which make up the entire living world of today are the same identical atoms which formed the living world of a million years ago, and that our own body may consist of some of the identical atoms which once were part of a dinosaur, or a tree fern of the coal age, or of one of our own ancestors. Only the pattern has changed, but the material from which the organic world has been formed, as long as there has been an organic world, has always remained the same. From dust to dust, as the Bible says; the same clay cast in ever-changing molds.

Once the importance of these cycles is realized, once we understand that life on earth can be maintained only by a continuous rotation of certain elements, it is clear that the continuity of life on earth depends upon bacteria. Without them, there would be no rotation, and if life had ever started on earth, it would have come to an end long, long ago.

Chapter Ten

BACTERIA HELP THE CITY FOLKS

The role of bacteria in the "dust to dust" cycle is as important to our own existence as to all other life on earth, and their work fits our needs perfectly. In fact, sometimes we wish that they might even work a little faster and produce their "dust" quicker. This is especially true when we think of the trouble and expense of sewage disposal. A single family on a farm has not much trouble getting rid of excreta and garbage, but for large cities, it becomes an engineering problem of major importance.

Two different problems must be solved simultaneously. Sewage represents a danger to the health of the community because the bacteria of certain intestinal diseases such as typhoid fever, dysentery, and even cholera, might be spread by it. Sewage presents also a great nuisance problem because it will produce very offensive odors unless its decomposition is carefully controlled. It is a historical fact that Parliament in London had to be dismissed one summer because of the intolerable stench of the Thames river.

As in many other cases, empirical knowledge had developed usable methods before the indispensable role of bacteria in sewage decomposition was understood, but some of these methods were not very satisfactory, and all of them have been improved with increasing knowledge of bacteriology. When in 1842, a large part of the oldest section of Hamburg burned down, the city engaged the famous English engineer Lindley to build a sewage system which was for a long time the pattern for all other cities. London was not so fortunate, for political reasons, because by far the largest part of the population lived in suburbs outside the very narrow city limits of London. Not until the continued cholera epidemics of 1847, 1849 and 1852–54 took tens of thousands

of lives, was a comprehensive plan for the drainage of greater London conceived, and this plan did not materialize until 1859. John Phillips testified in 1847:[1]

> There are hundreds, I may say thousands, of houses in this metropolis which have no drainage whatever, and the greater part of them have stinking, overflowing cesspools. And there are also hundreds of streets, courts and alleys that have no sewers, and how the drainage and filth are cleaned away and how the miserable inhabitants live in such places, it is hard to tell.
>
> In pursuance of my duties from time to time, I have visited very many places where filth was lying scattered about the rooms, vaults, cellars, areas, and yards, so thick and so deep that it was hardly possible to move for it. I have also seen in such places human beings living and sleeping in sunk rooms with filth from overflowing cesspools exuding through and running down the walls and over the floors. . . . The effect of the effluvia, stench, and poisonous gases constantly evolving from these foul accumulations were apparent in the haggard, wan, and swarthy countenances and enfeebled limbs of the poor creatures whom I found residing over and amongst these dens of pollution and wretchedness.

That was the situation in the largest city of the world just 100 years ago. Great progress has been achieved because engineers and bacteriologists have cooperated to make the bacteria mineralize the organic matter of sewage in the shortest possible time, and to change sewage to an inoffensive, pure water without any danger to health.

The problem lies primarily in the enormous quantities of water involved. The average volume of sewage in American cities is 100 gallons per capita per day. This means that a little town of 20,000 inhabitants must dispose of two million gallons of sewage every day, and for a city of half a million people, it amounts to 50 million gallons.

This huge volume of water must be so purified:

that it does not cause a nuisance;
that it is not injurious to aquatic life;
that it is no longer a danger to health.

[1] Quoted from Metcalf & Eddy, Sewerage and Sewage Disposal; McGraw-Hill Book Co.

To get an idea of the task of purification, we must first know how dirty sewage is, and the sanitary engineer will tell you upon inquiry that sewage is not dirty at all. He smiles when he tells the standing joke that sewage is really very pure water, 99.94% pure. That sounds as if we could not wish for any better water. But the 0.06% impurities certainly cause a good deal of trouble.

This figure of 0.06% impurities in sewage is an average for sewage from residential towns. Factories may change the sewage composition greatly. Creameries, canning factories, tanneries and slaughterhouses bring much more organic matter into sewage than residences. Large factories of this type must purify their sewage before being permitted to run it in the city sewer. Frequently, they have sewage disposal plants of their own.

It may seem surprizing that such a small amount of impurities requires a special sewage disposal plant with engineers and machines and large areas of canals, filterbeds and even glass houses. The reason for all this equipment lies partly in the large quantities of sewage to be handled, and partly in the nature of the impurities in the sewage. If no large factories empty their wastes into the city sewer, the organic matter of the sewage consists largely of excreta, kitchen refuse, soap and paper. The excreta represent the indigestible part of our food, and the kitchen refuse comes largely from the non-edible parts of our food. Hence the organic matter in sewage is hard for bacteria to digest, and decomposition is slow.

The chief trouble, however, lies in the insolubility of the oxygen. Mineralization means complete oxidation. As soon as bacteria start to decompose sewage, they use up what little oxygen there was dissolved, and then anaerobic putrefaction sets in with its horrible odors, and the dreaded nuisance is established. The bacteriologist has worked out a method by which he measures how much oxygen a certain sewage needs. This is called the Biological Oxygen Demand,

and is expressed in parts of oxygen per million parts of sewage (ppm). This amount averages:

For raw sewage	About 140 ppm
For settled sewage	About 90 ppm
For canning factory sewage	About 1,500–2,000 ppm, up to 3,000 with corn canning
Solubility of oxygen in water	8–10 ppm

Here is the dilemma. Oxygen is soluble only to the extent of 8 to 10 ppm, and sewage needs at least 10 times as much. Of course, the oxygen supply in the air is practically unlimited, and more oxygen will dissolve when the original dissolved oxygen of the sewage is removed by bacteria. But this process of dissolving is slow, and the process of removing is rapid. We have already become acquainted with the amazing appetites of bacteria which not only consume their own weight of food every hour, but require a corresponding amount of oxygen to produce complete mineralization.

The old way out of this trouble was to run the sewage in a river. That dilutes the organic matter, and the water in the river usually contains a fair amount of dissolved oxygen. We can make an accurate calculation of how this works out, depending upon the ratio between water and sewage. Let us assume one million gallons of sewage per day, with an oxygen demand of 90, which makes the total demand 90 million units. This sewage runs into a river flowing at the rate of 15 million gallons per day, and containing 8 ppm dissolved oxygen, thus providing 120 million units, which is 30 million units more than is required. We are certain, therefore, that there will be no nuisance.

However, this is not sufficient to protect the aquatic life. The oxygen requirements of fish vary with the species, and with temperature, but it is usually assumed that at least one-third of the maximal solubility of oxygen must be present to prevent the fish from suffocating. That means we should see to it that at least one-third of 8–10 ppm, or

about 3 ppm of oxygen are left in the river. What we actually have in our example is 30 million units of oxygen in 16 million gallons of water, and that is not quite 2 ppm of oxygen. In this sewage-polluted water, all fish would die.

The amount of sewage that can safely be dumped into a river depends upon many different factors. There is to be considered the kind of sewage, especially its oxygen demand; there is the water whose oxygen content may be considerably below normal on account of recent contamination further upstream. The rate of flow and the depth of the water are important. Then, of course, comes the ratio of water to sewage. No general United States standards have been established, probably because of too many uncertain factors. But it is generally assumed that the sewage of 1,000 inhabitants requires a river with a water flow of at least $3\frac{1}{2}$ to 6 cubic feet per second. This is identical with 2.2 to 3.9 million gallons per day, and if this is sufficient for the 100,000 gallons of sewage from 1000 people, it means that there must be about 22 to 40 times as much water as sewage. In our example above, we had assumed only 15 times as much water.

When the sewage flows into the river, the river becomes turbid. Some of that turbidity will settle to the bottom unless the river flows quite rapidly, and this sludge turns black from anaerobic decomposition. It causes no nuisance as long as the water above has enough oxygen. Small, red sewage worms will eat the sludge and thus help in the sewage disposal. The intestinal bacteria cannot develop in this impure water; they may remain alive for a while, but after a day or so, they begin to decrease, and in 5 to 7 days, over 99% of them have completely disappeared. They play no role in purification. Mineralization is accomplished by quite a different group of bacteria which are found only in small numbers in the intestine. Many of them come from the river water. Where the sewage is mixed with the water, they are present only in small numbers, but with the sudden

increase in their food supply by sewage, they multiply rapidly and reach their maximum usually in less than 24 hours. By this time, the river has flowed many miles. Here where they are so numerous, they decompose the organic matter of sewage rapidly and thereby use much oxygen. It is at this stage that the oxygen supply becomes critical, not at the sewer outlet. If there is not enough oxygen, foul odors are given off, especially in summer, water plants and animals die, the bottom of the river changes to a dismal, slimy black, and dead fish float on the surface.

Ultimately, of course, air will be gradually dissolved in the flowing river, and finally, the anaerobic condition will be overcome, and further down the river, plant and animal life will again be able to exist.

When the water supply of the river is at least 25 times that of the sewage, such conditions cannot develop. The bacteria will continue to decompose all organic matter from the sewage, the water becomes clear, water plants saturate the river with oxygen, and finally, even the ammonia is changed to nitrates, as the final step to complete mineralization.

By this time, all food for the bacteria is exhausted and they begin to decrease in numbers, partly by settling out, partly by being eaten by protozoa and other microscopic animals which in turn are the food for larger water animals. The rest die from starvation or from the ultraviolet rays of the sun which can penetrate again into the clear water. Thus, the river has finally purified itself. The water is again clear and sparkling, full of oxygen, free from bad odors, bad tastes and from pathogenic bacteria which died with the other intestinal bacteria. We should not forget that this so called "self-purification" of the river is not accomplished by the river, but by the bacteria.

Where conditions do not permit the dumping of sewage directly into a river, lake or ocean, we can try to make the best of anaerobic putrefaction. The simplest case of this type is the septic tank for single farms or very small communi-

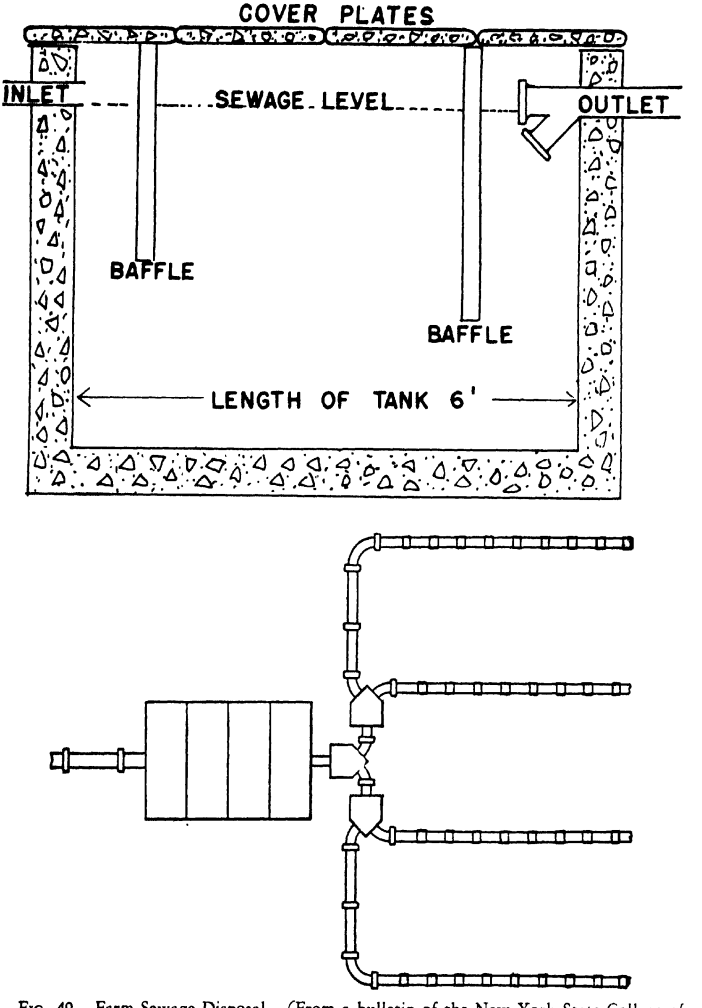


FIG. 49. Farm Sewage Disposal. (From a bulletin of the New York State College of Agriculture; Courtesy of Professors H. W. Riley and J. C. McCurdy.)

Above: Cross section of a small septic tank.

Below: Top view of drainage system carrying off the overflow from the tank to the land.

ties. It consists of a large concrete tank, entirely underground, into which the sewage runs. The sludge settles, the fat and other floating particles form a skum, and the decomposition underneath becomes completely anaerobic. Gases are formed, but they escape through drainage tiles into the ground and do not become noticeable. The size of the tank is so adjusted that the sludge decomposes at the same rate as it is formed, so that cleaning is necessary only once or twice a year to remove mineral and other matter that

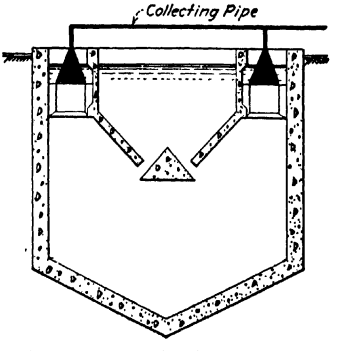


FIG. 50. Cross section through an Imhoff tank. The blackened part is a gas collection equipment. (From Metcalf & Eddy: Sewerage and Sewage Disposal. Courtesy of McGraw-Hill Book Co.)

does not decompose. The run-off is underground. The partly decomposed sewage runs into branching drainage tiles which are laid in sand or gravel. The sewage trickles and seeps through and between the tiles into the sand where it is completely oxidized.

Similar to this is the Imhoff tank for large communities. The sewage flows slowly through a large open tank, the upper middle part in our cross section, and the sludge settles and falls through slits into a separate chamber. There it putrefies without disturbing the flow and sedimentation because the putrefactive gases escape through a separate opening at the side of the tank. Our illustration shows how these gases, consisting mostly of hydrogen, methane and

carbondioxide, may be collected and used for heating purposes. The sludge chamber has to be cleaned only twice a year, because the organic matter decomposes almost as fast as it settles out. The effluent of the Imhoff tank still contains a good deal of organic matter, and is usually treated further.

In recent years, it has become customary to separate the sludge from the rest of the sewage, and to treat the two sections separately. The separation is accomplished by letting the sewage flow slowly through a wide basin where the suspended particles can settle. About one-third of the organic matter can thus be separated from the large volume of sewage. The sludge amounts only to about a quart per person per day, and this amount can be handled like chemicals in a chemical factory. The effluent from such settling tanks contains still 200 ppm organic matter. In some cities, this is reduced further by chemical precipitation by means of alum, or ferrous sulfate and lime.

If this settled sewage with its smaller oxygen demand cannot be dumped into a lake or river, it must be further purified. Bacteria are the only means by which this can be done. We have seen that fresh sewage does not contain the right oxidizing bacteria in sufficient numbers, and that fastest decomposition does not take place immediately after mixing with river water, but some twenty hours later, much further down-stream. And then, all these bacteria which had done such splendid work, die from starvation. That is a great waste of energy and time which Nature can well afford, but which we must try to avoid. Thus, our sewage purification systems employ the same bacteria over and over again, by making them stationary and letting the sewage flow past them.

The simplest process is to pump the sewage on sand. Some cities are situated in sandy areas, and they can run their sewage without special provision onto the sand which thereby is not only kept moist, but also fertilized. This

method of disposal has been developed into sewage irrigation farms. Berlin, the capital of Germany, lies in a vast area of very unfertile sand, and it has a sewage farm of 43,000 acres. Paris irrigates 12,000 acres. England has 8 experimental sewage farms.

From this experience developed the intermittent sand filter for cities which are not situated in sandy areas. Sand beds with good natural or artificial drainage are carefully prepared, at the rate of one acre for about 500 to 1500 people. The sewage is distributed over the sand, and soon, every sand grain will be coated with bacteria which oxidize the

FIG. 51. Schematic drawing of sewage irrigation. (From Lafar. Technische Mykologie, Vol. III)

organic matter. When the flow of sewage is stopped, air is drawn into the lower strata by the receding water, and thus, by alternating flow and stop, anaerobic conditions are entirely avoided. The intermittent sand filters are very efficient, and the effluent is very clean.

Another common method of sewage oxidation is the contact bed. This consists of long cement basins, 4 to 6 feet deep, filled with broken stone or slag. Porosity of the filling material is important because it must catch as much oxygen as possible. The sewage is slowly run into the basin until it is full. The stones are covered with thick layers of oxidizing bacteria, which have had time to store oxygen, and the pores of the stones also are saturated with it. Thus, oxidation of the sewage can go on under optimal conditions, until after a few hours, all the oxygen is used up. Then the partly oxidized sewage runs off, eventually to another, simi-

lar bed, and the bacteria are given about 6 hours time to load up with fresh oxygen. Then the next batch comes in. One acre of contact beds takes care of the sewage of about 4,000 to 8,000 people, but the effluent is not as good as from a sand

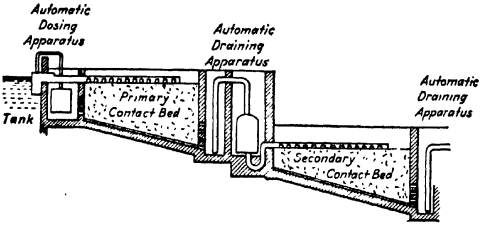


FIG. 52. Double contact bed. (From Metcalf and Eddy: Sewerage and Sewage Disposal. Courtesy of McGraw-Hill Book Co.)

filter. A better result is obtained by double contact beds, but they take more space.

Similar to the contact bed is the trickling filter which consists merely of a high mass of broken stone or cinders or similar coarse material over which sewage is trickling con-

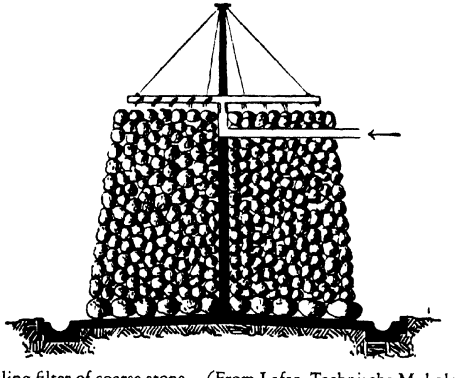


FIG. 53. Trickling filter of coarse stone. (From Lafar, Technische Mykologie, Vol. III.)

tinuously. As there is continuous contact with the air, the sewage decomposition is oxidative. The surface of the stones is covered with a thick mass of slime, so thick that frequently big chunks break loose and float away, and must be taken care of. For large communities, very elaborate

mechanisms have been constructed to distribute the flow of sewage uniformly over the filter surface. Spraying is the preferred method.

In all these filters, the bacteria are attached to sand or rock particles and remain stationary while the sewage flows by. In this way, the bacteria are used to maximum efficiency. Quite different is another way of making the same bacteria do more work. In the "activated sludge" process,

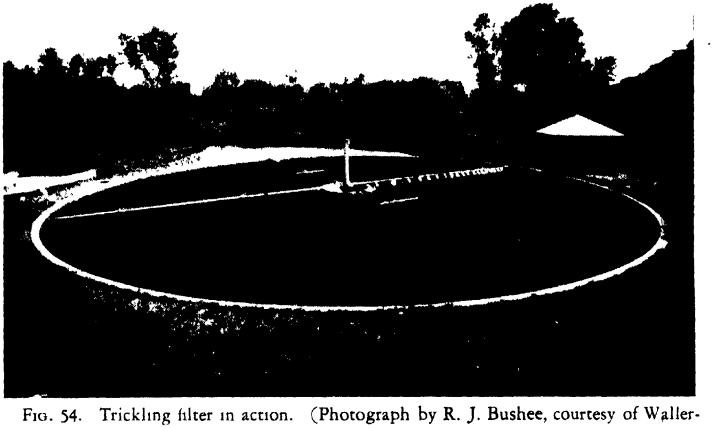


FIG. 54. Trickling filter in action. (Photograph by R. J. Bushee, courtesy of Wallerstein Laboratories)

the aeration is accomplished by blowing air through the sewage, and the bacteria are made to do more work by letting them settle out from the oxidized sewage, and then pumping them back into the fresh sewage where they can start their work all over again. Aeration is usually accomplished by forcing compressed air through pipes with many holes which are at the bottom of the sewage canal. After aeration, the sewage flows through a settling basin. Here, most of the bacteria sink to the bottom, forming a sludge which is scraped together into pits, and some of this sludge is pumped back and mixed with the fresh sewage. The sewage is thus inoculated with large amounts of very actively

oxidizing bacteria, and these, with the fresh food supply from the fresh sewage and the plentiful oxygen supply from aeration, will multiply and attack the sewage rapidly. They are continually circulated, and owing to the unusually large numbers of bacteria present, the result is very good. However, when a new activated sludge system is started, it takes nearly two weeks to get the right kind of bacteria in sufficient numbers. Often, sludge from a similar system in another town is hauled in tank trucks to inoculate the fresh sewage.

The effluent from sand filters, contact beds, trickling filters and aerated tanks is usually sufficiently decomposed to permit its being dumped into a river or lake without danger from offensive odors. The Biological Oxygen Demand is greatly reduced. If that is not the case, an additional sand filter or trickling filter will purify the sewage still further. It is not unusual to find the protein of sewage broken down by sand filters not only to ammonia, but to nitrate which is the utmost degree of mineralization.

Sludge Disposal: All these different methods of purification are undertaken with settled sewage, and we must now discuss what to do with the sludge. The amount of sludge is relatively small, only about a quart a day per person. The sludge is quite concentrated, contains about 4% solids, and is a black, thick, liquid mass of no very offensive odor because it has not as yet started to ferment. There are several primitive disposal methods. It can be run unto sand which lets the water pass through, but keeps the solids back which will slowly dry and can be used as filling material, or fertilizer, or can be incinerated if very dry. When the drying is slow, on rainy days, the odor will become very foul.

As the sludge, when left to itself, ferments with the evolution of combustible gases, it has been found practical to let the sludge undergo fermentation carefully controlled in regard to acidity and temperature. The gas produced in this way is usually sufficient for the heating and lighting of the sewage plant, and sometimes even furnishes the power

to run the sewage pumps. The fermentation lasts about a week. The residue still contains a large amount of unfermentable organic matter. Only about half of the organic materials of the sludge are changed into gas. The rest is dried as was described above. To avoid trouble through wet and cold weather, the drying is usually done in glass houses.

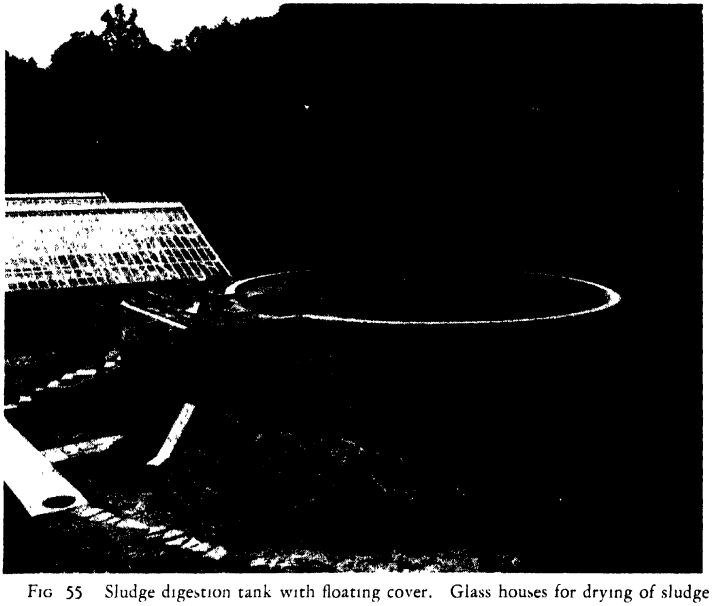


FIG 55 Sludge digestion tank with floating cover. Glass houses for drying of sludge in background. (Photograph by R. J. Bushee, courtesy of Wallerstein Laboratories.)

A very special problem is presented by the canning factories whose sewage is about ten times as concentrated as that of residential towns. Besides, many canneries work only a few months of the year, so that it would not seem worthwhile to build an elaborate sewage system. On the other side, these factories work during the hottest months of the year when the nuisance from sewage is most pronounced. A novel solution has been recently proposed

which again makes use of certain properties of bacteria. As will be pointed out in the next Chapter, certain bacteria are so avid for oxygen that they will take it from other chemical compounds such as nitrates if the oxygen from the air is no more available. It is certainly preferable to have the bacteria be satisfied with nitrates rather than start an anaerobic putrefaction. An attempt was made to satisfy the oxygen hunger of the bacteria by putting plenty of nitrate of soda into the sewage, with the result that foul and offensive odors could be prevented completely. While this process of treating sewage is fairly expensive, it costs not nearly as much as the installation and maintenance of an elaborate sewage disposal system.

Chapter Eleven

BACTERIA HELP THE FARMER

As long as plants have grown on earth, they have grown more luxuriously in some places than in others. Many factors influence plant growth, such as temperature, rainfall, humidity of the air, length of daylight, etc. But even when all climatic factors are identical, the differences in plant growth may still be very great because of different soil fertility. Some of these differences have been caused by man who has robbed the soil of some important plant foods, but even before the advent of man, soils varied greatly in their fertility and they contained different amounts of plant food, because they originated from different rocks. These original differences were aggrevated in localized areas by the activity of animals, man, and rain.

The total organic matter of the world does not change much in a century, and it is safe to say that all organic matter of today is sufficient to produce all the organic matter of tomorrow, provided that all of it is maintained in circulation. However, even if the total organic matter of the world would rotate continuously, the soil fertility of an individual plot of ground may still change. A herd of buffalos migrating over the prairies, or a swarm of locusts settling on a piece of ground may eat up every blade of grass in that place, and then migrate on, dropping their excreta elsewhere, and dying and rotting in still another place. All elements of the eaten grass return to circulation, but at different places, and the one piece of ground is definitely depleted in fertility while another plot has gained.

Another source of local loss is drainage, and this is more serious because it is not temporary, but continuous. Most soils, especially those with a good humus content, have the ability to hold the mineral plantfoods, such as potassium,

lime, and phosphate, by adsorption which prevents their being washed out by rain and carried into the subsoil. With prolonged, heavy rainfalls, only a slight amount of the soluble minerals is leached out and carried down to the ground water, and from there into a creek, and finally into the ocean. This local loss is usually made up by a slow weathering of the rock particles of which the soil consists. Ammonia also is absorbed by the soil, but nitrate is not. This most important plant food is thus rather easily washed out into the subsoil with every heavy rain, and the soil loses part of its fertility. It is not a great part that is lost at one time, because soil never contains very much nitrate. Sometimes it contains none at all, and very rarely will it have more than 0.01 percent of the total soil weight.

Even a complete washing out of all nitrate would not deplete the soil of its entire nitrogen content. All soils contain organic matter in various stages of decomposition, and for farm soils, the organic matter averages not less than 2 percent of the soil weight. At least one-twentieth of this is nitrogen. That would be an average of about 0.1% nitrogen of which very rarely more than one-tenth is present in form of nitrate. The soil has thus a considerable backlog of nitrogen which cannot be leached out by rain, and which is decomposed only very slowly into nitrate. Nitrogen is usually considered the most precious of all plant foods, because its amount is so limited, for it is not part of the rock like calcium, potash or phosphate. On the other hand, it is needed for making proteins which are the substrate and mainspring of all life activity. Loss and gain of nitrogenous matter seems therefore more important than that of any other element.

While all soils lose nitrogen through rain, they also gain nitrogen through rain. By the electric discharges in the atmosphere, nitrogen, oxygen and water are combined in very small amounts to ammonium nitrate which comes down with the rains. This gain of nitrate may balance the

loss through drainage, or it may not balance it. That depends upon many factors, and it need not be discussed here because bacteria have nothing to do with it.

More important than the gains and losses of soil nitrogen by rain are the gains and losses brought about by certain groups of bacteria, some of them growing and multiplying in the soil, others living in the roots of plants, in a very interesting association where it is difficult to decide whether the bacteria are parasites of the plants, or the plants are parasites of the bacteria.

It may seem odd that as big a thing as a green plant can be a parasite of so small a thing as a bacterium. Of course, it all depends upon the definition of the term parasite. The pitcher plant is a parasite of insects; it catches them, kills them and digests them. The legumes which are parasites of certain bacteria, do their job more cunningly. They lure them to their root hairs, and if the bacteria get in, they are held prisoner, and are well fed with sugar, but they are robbed of their protein. With this source of nitrogen from the bacteria, the legumes can get along with very little nitrate in the soil, and even without any nitrogen at all as our picture shows. Fortunately these bacteria have the ability to use the nitrogen gas of the air for making their protoplasm. This is a very unusual property. All other plants, animals or microbes must get their nitrogen for cell construction in form of nitrates or ammonia or amino acids. No organism can use nitrogen gas except three small groups of bacteria, the Rhizobium (Greek for root-growing organism) which lives only in legumes, but in no other plants, and two other groups of bacteria which will be discussed later in this chapter.

As long as the plant furnishes enough sugar, the bacteria will use this together with nitrogen from the air to make their own protein and to grow and to divide and multiply, although the plant takes its toll of the newly-made protein. The multiplying bacteria press from the inside, and irritate

the roothair which swells and grows. Really, the roothair becomes a small tumor and is called a nodule. On a clover root, it is only about as large as a pinhead, but on peas, beans and vetches, it may be larger than a pea. The plant is capable of getting part or all of its nitrogen supply from these bacteria, and it is in the interest of the plant to keep the bacteria alive. It must not kill the goose that lays the

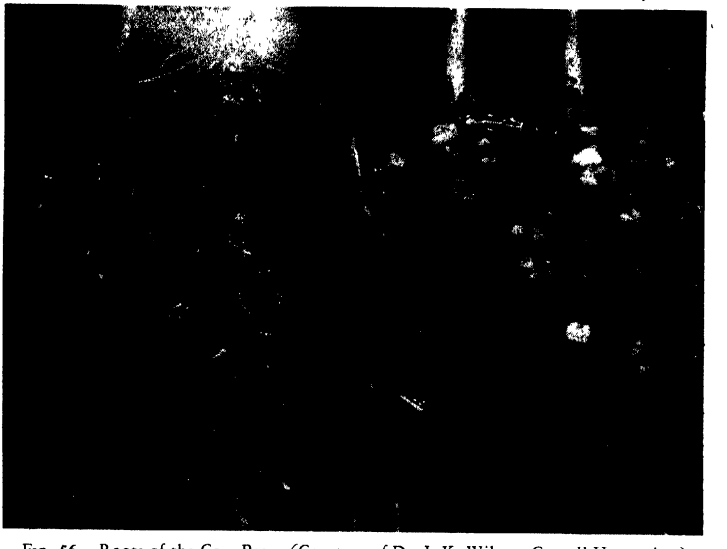


FIG. 56. Roots of the Cow Pea. (Courtesy of Dr. J. K. Wilson, Cornell University.) Left: uninoculated. Right: inoculated, with many nodules.

golden eggs. The legumes are the only plants capable of keeping these bacteria as prisoners in their roots, and on account of this good nitrogen supply, they contain much more protein than other plants. Every farmer knows the greater feed value of clover or alfalfa hay over grass hay or corn silage for his livestock, and every housewife—or at least every home economics teacher—knows that beans, peas and lentils, and the so greatly advertised soy beans, contain so much protein that they can be used as meat substitutes. The excess of protein in these plants is due to the labor of the nodule bacteria which "fix" the nitrogen of the

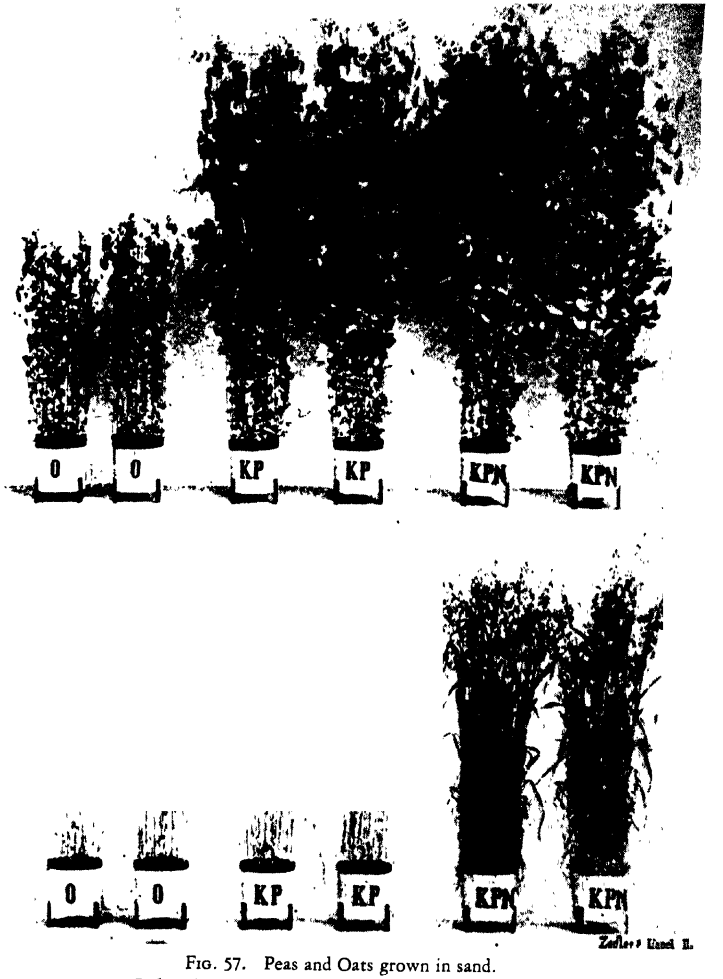


FIG. 57. Peas and Oats grown in sand.
Left: without any fertilizer.
Middle: with potassium salts and phosphate.
Right: with potassium salts, phosphate, and nitrate.
The K P fertilizer without nitrate did not help the oats at all while the peas grew almost as well without as with the nitrate fertilizer. (From Lafar, Technische Mykologie.)

air to make protein. The term "fixing" is an old alchymist term, meaning here that the invisible nitrogen gas is bound into chemical compounds which can be seen. The Rhizobium is called a nitrogen-fixing bacterium.

If we consider that the bacteria, although in captivity, lead a rather sheltered life and can multiply, and are well fed, it may not seem quite right to call the legume a parasite. After all, the bacteria outside the plant root would have a very frugal existence in the soil. But so would the cows and pigs if we would not care for them and feed them and shelter them. And we are certainly the main parasite of cows and pigs,—if the above definition of a parasite is accepted. However, since this is not a textbook, we can enjoy the facts of life without squabbling about definitions, and the important fact is that in the soil, these nodule bacteria die more rapidly than they can multiply. As a rule, the soil from a field of peas contains quite a good number of the pea Rhizobiums after the peas are harvested. The nodules of the dead plants rot and set the bacteria free. But a year later, there are fewer Rhizobiums in the soil, and after two or three more years, only very few have managed to survive.

That is important to the farmer. He wants the peas and beans in his fields, the clover and alfalfa in his pasture to have plenty of nodules because that saves fertilizer, and guarantees a good crop. But the number of nodules cannot be large if the number of bacteria in the soil is small. There is only one way to relieve this situation, and that is to furnish the bacteria.

The Rhizobium can be readily grown in pure culture on a specially prepared agar, and such a culture, containing several billion bacteria, can be mixed with the seeds just before sowing. When the seed germinates, the bacteria are very close to the tiny rootlets, and the chances are pretty good that a few of the hundreds of hairs on the roots become infected with a Rhizobium, and develop a nodule.

Different species of legumes have different species or at least different strains of nodule bacteria. The agricultural experiment stations and some commercial laboratories have made extensive experiments to find cultures which are especially efficient in nitrogen fixation. This has proved very successful, and inoculation of legume seeds with pure cultures is now a general farm practice.

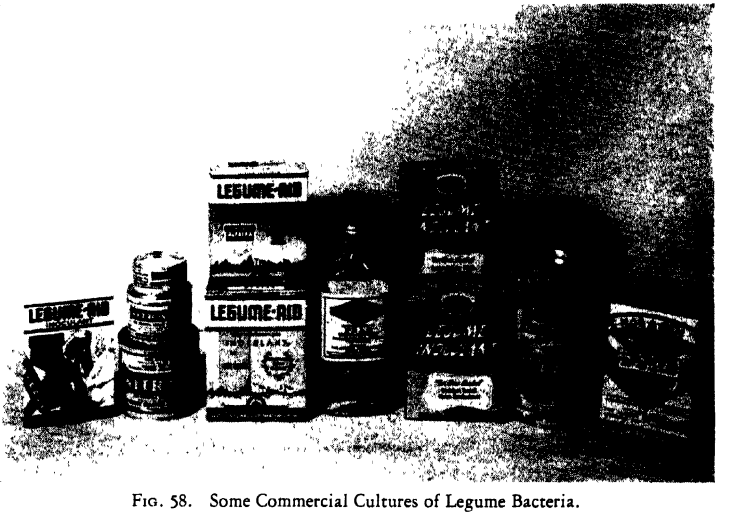


FIG. 58. Some Commercial Cultures of Legume Bacteria.

The nodule bacteria are not the only bacteria that can fix nitrogen. Another group deserves equal credit; it consists of a very few species which have been named *Azotobacter* (photograph in Chapter One). They live in the soil in fairly large numbers, if the soil suits their needs. They too must have sugar, or some similar good food, because it takes a lot of energy to fix nitrogen. But the Azotobacter does not get caught in plant roots. It grows free in the soil, feeding on the remains of dead plants like most other soil bacteria. It is easily grown in pure culture. In a solution containing only sugar and minerals, in which no other plant, animal or microbe can grow for lack of nitrogenous

food, *Azotobacter* grows luxuriously and produces large quantities of cells. It has been shown recently that in case of great protein scarcity, protein could be made from sugar and air by growing this bacterium in dilute molasses and phosphate in large tanks, just as bread yeast is grown. The bacteria are good food, just as good as yeast, and they can be grown without ammonia which is necessary for the yeast.

Azotobacter is found in almost all soils which support plant growth. It needs lime, and will not develop in acid soils. It has one other most peculiar need, namely molybdenum, a rare mineral. Only very small amounts are required, but Azotobacter will not grow when molybdenum is entirely absent. No other organism so far has been found to depend upon this rather rare element. Azotobacter is found in all parts of the world.

Another type of nitrogen-fixing bacterium belongs to the anaerobic group of Clostridium. These bacteria may not be important in light sandy soils which are well aerated, but they are found in every soil, all over the world; they are even more frequently present than Azotobacter. In clay soils which are easily waterlogged and subject to frequent anaerobic conditions, they may be quite important.

While these last two groups are of great importance to soil fertility, the farmer cannot do very much about them. If the soil is fit for them, they are already present and doing their work, and if the soil is not suited, it would be useless to inoculate the soil with them because they would die. Only in a few instances can the soil be improved so that the Azotobacter will be able to multiply, as e.g. by liming a soil that is too acid.

This nitrogen fixation by Rhizobium, Azotobacter, and Clostridium upsets the nitrogen cycle described in the Chapter From Dust to Dust. According to that discussion, the amount of chemically bound nitrogen is limited, and circulating all the time, from plants to animals to ammonia to nitrate and back to plants. This means that the total amount of life on earth is limited by the amount of bound

nitrogen. There is plenty of free nitrogen, four-fifths of the entire atmosphere around the earth, but that is not available for plants and animals. Nitrogen is a very inert gas.

The nitrogen-fixing bacteria are the only exception to this cycle, and as they are doing their job in almost every ounce of soil and in the roots of every leguminous plant, the amount of bound nitrogen on earth is continuously increased by them. If it were not for one leak by which bound nitrogen is set free again, the total amount of plants and animals on earth would increase rapidly from year to year.

This leak which sets the bound nitrogen free again is also caused by bacteria. In our study from dust to dust, we have seen that the ultimate stage of the mineralization of proteins is carbon dioxide, water and nitrate. In well fertilized soils, a fair supply of nitrate is available. After heavy rainfalls, the soil is waterlogged. All its pores are filled up with water; no air can get in; the little oxygen dissolved in the soil water and held in air bubbles is soon used up, and conditions become anaerobic. The angle worms can get no air and crawl to the surface. Bacteria also get no air, but they cannot crawl to the surface. If they cannot get the oxygen from the air, they try to get it from those substances which contain a good deal of it. The nitrate molecule contains three oxygen atoms for each nitrogen atom, and many bacteria can use the oxygen of the nitrate molecule for their respiration. Thereby, they change the nitrate chemically; some change it to nitrite, others change it to ammonia. Neither of these changes is a real loss because as soon as the water has run off and air gets back into the soil, the nitrite and nitrate bacteria will change these compounds back to nitrate. But there is a third group, called the "denitrifying bacteria," which take every bit of oxygen out of the nitrate molecule and leave only nitrogen gas. This simply goes into the air and is a complete loss. Denitrifying bacteria are found in every soil, and in the rivers, lakes and oceans. They can do their destructive work

only under special conditions, namely when no air is present. It is impossible to estimate the amount of bound nitrogen which is set free by them every year. It is probable, however, that more nitrogen is fixed on earth than is freed.

Even if the balance between nitrogen fixation and denitrification results in a slow increase of bound nitrogen, the increase is too slow to offset, in one year, the depletion caused by a buffalo herd or a grasshopper swarm. As a rule, such events occur only infrequently, and the large backlog of humus in soil permits new and fairly normal plant growth in the following year. More dangerous to soil fertility is man who removes large quantities of organic matter containing carbon, nitrogen, potassium and phosphorus, year after year from the same field. If these precious elements are not replaced, the soil becomes depleted, and will bear smaller and smaller crops every year. The soil is robbed, as the saying goes.

The conscientious and intelligent farmer replaces the elements which he removed. Fortunately, he need not replace the carbon which constitutes the major share of the removed elements. The plants take their carbon exclusively from the carbon dioxide of the air, and wind and weather see to it that all the carbon dioxide produced in the cities goes back to the country. The other elements are usually replaced either as manure from the farm animals, or as "chemical fertilizer" in the form of calcium phosphate, potassium chloride, sodium nitrate, or ammonium sulfate.

The original and complete fertilizer of the farmer is, of course, the barnyard manure. Manure contains some potassium, some phosphorus, some ammonia and urea and some nitrogen in insoluble form. Ammonia, urea and part of the insoluble nitrogen is readily changed by bacteria to nitrate. The rest remains quite insoluble and increases the humus which can be decomposed but very slowly. Manure also contains straw, and the proportion between straw and excreta is important. If the manure contains very much

straw, it may have no direct fertilizing effect at all. Straw consists of many different kinds of carbohydrates all of which are digestible for certain bacteria. These bacteria will multiply readily if much straw is available. They use the straw for respiration, but in order to grow, they must have some nitrogenous matter, and so they use the ammonia, urea and other soluble nitrogenous compounds of the manure to build up their own cells. Thus, the manure acts as fertilizer for the growth of bacteria, which multiply to enormous numbers in a few days, if there is much straw, and no nitrogenous food may be left for the much slower-growing crop plants. A soil can be made absolutely sterile, incapable to support any plant growth whatever, by mere addition of finely powdered straw or paper. Of course, this is only temporary. The nitrogen of the manure has not been destroyed, nor has it disappeared. It has only been changed into a form in which the plants cannot use it, namely into bacterial bodies. When all food is used up, the bacteria will gradually die from starvation, their dead bodies are decomposed by other bacteria just as all other dead organic matter is always decomposed, and ultimately, after many transformations, the nitrogen will appear again as nitrate.

The bacteria are not evenly distributed in the soil because the soil itself is not at all uniform. It consists of about 98 parts of rock in various stages of disintegration, and of only about 2 parts of organic matter, largely humus. Humus is not really a well defined chemical compound, but a mixture of many very different, insoluble substances. As leaves fall down upon the soil, as rain washes some of the leaf particles into the lower strata, as the soil surface dries in the sun, as a big angleworm or a tiny nematode digs its way through the soil, making a channel for good ventilation and leaving a track of excreta behind, there is a continuous change going on. Every inch presents different conditions, has a different "soil climate," and the soil bacteria vary accordingly.

Most of the time, the soil bacteria are hungry, starving,

remaining alive merely because they have become accustomed gradually to long periods of starvation. Then a leaf falls suddenly upon the ground, providing a large supply of starch and cellulose, and the soil population has a feast for several days. Starch is easily digested by many species, and in the immediate neighborhood of the leaf, bacteria multiply hundredfold and thousandfold. The species that digest cellulose are slow-growing, and their feast lasts longer. Other groups like the nitrate or sulfur bacteria are not affected at all by this blessing because none of the leaf constituents means food for them. When this food supply is exhausted, most of the newly-grown bacteria which were born in an era of abundance cannot survive the sudden scarcity; they die. Relatively few can adapt themselves again to the hard times and remain in a semi-dormant state dreaming of new supplies to come.

As all dead matter normally falls on the surface, and as it is largely insoluble, most decomposition takes place on the surface, and only in the top soil do we find very large numbers of bacteria. Relatively few are found below the first foot of soil. Lack of oxygen in the lower strata also tends to decrease the population. Some idea of the numbers involved may be obtained from the following figures, taken from Waksman's book on Soil Microbiology, which are representative of the numbers of bacteria found in different types of soil at different depths. The numbers indicate bacteria per gram of soil.

	Garden soil	Pasture soil	Forest soil
Top inch	7,000,000	10,000,000	2,000,000
4 inches below	7,500,000	5,000,000	1,000,000
8 inches below	4,000,000	3,000,000	500,000
12 inches below	1,500,000	1,000,000	300,000
30 inches below	300,000	200,000	100,000

Six feet below the surface, the soil has sometimes been found to contain a few thousand bacteria, but in other cases, it was completely sterile, free from any bacteria. Fence

posts do not deteriorate at the tips which are deep in the ground, but within the first two inches from the surface. The old palaces of Venice are built on large numbers of piles rammed into the swampy ground which in itself did not offer sufficient firmness to support the weight of the massive stone buildings. After 500 years, these piles still support the palaces. No deterioration has taken place so far beneath the surface. The same phenomenon has been used to explain the formation of coal. Floods may have swept whole forests into lakes where the entire mass of plants sank down, was covered with mud which kept the oxygen out, and thus escaped complete destruction by bacteria.

Soil is not really necessary for plant growth. During the last decade, the magazines brought lengthy articles about hydroponics, or the art of growing plants in water, without soil. To plant science, nothing was new about this hydroponics except the impressive name. For more than a century, plant physiologists have been growing plants in water for experimental purposes. However, the vast majority of plants do grow in soil, though ponds, rivers, lakes and oceans produce a flora all of their own. The water culture of plants was largely a fad, but it has its place in a few isolated cases where cost does not matter. Hydroponics is a fine illustration of the two main objects of soil: to provide a firm support for the plants, and to provide food for the plants. In order to grow plants in water, we have to furnish mechanical support for each individual plant, and that would be no easy task for a few acres of corn. Besides, we have to provide the plant food. The roots must not only be put in water which contains all the necessary minerals in balanced solution, but as the plants take up some of these compounds more readily than others, these compounds must be replaced; also, the roots must be aerated. In short, so much work is required for hydroponics on a large scale that it is far easier and cheaper to let the plants grow in soil and let the bacteria provide them with all the foods they need, by giving the bacteria the raw materials.

Chapter Twelve

THE 1941 CENSUS OF BACTERIA IN THE UNITED STATES

When I told my friends that I was taking a census of the bacteria in the United States, they ridiculed the idea. Some laughed because they thought it was useless, but it did not seem so to me for I had a very definite object in mind, namely the ratio between disease bacteria and useful or at least harmless bacteria. Others laughed because they thought it would be impossible. Really, they were correct because we can make only a very rough and inaccurate guess. Our estimate may be ten times too large or ten times too small, and one might question whether such inaccurate numbers would have any value whatever. Certainly, if the differences are to prove anything at all, they must be very great, and the final result will show that they *are* very great. The following estimates are given in considerable detail because this calculation has never been done before. Those readers not interested in following calculations may turn directly to the results summarized in the table near the end of this chapter.

Since these calculations were undertaken to emphasize the fact that there are more harmless and useful bacteria on earth than disease bacteria, all calculations were made in the following way: Of all harmless and good bacteria, a low estimate was made to be sure that there are more of this kind in the U.S. than our census indicates. Of all disease bacteria, the estimate was made so large that the actual number is certainly much less than our census indicates. If under these conditions, the good bacteria are still ahead of the bad ones, there can be no doubt about the predominance of good over evil in the world of bacteria.

At the very start, we run into one difficulty of vital statistics of bacteria, namely their enormous possibilities of

reproduction. Some bacteria multiply continually, day-in and day-out, for instance the intestinal bacteria. Others remain very stable and may multiply only a few times in a year, merely often enough not to die from old age, like tubercle bacteria or soil bacteria. The only way out of this difficulty seems to be to count the number of bacteria born each year. Most of them may not live to see the end of the year, but they have made their tiny impression upon the universe. This seems to be a fairer and more accurate method than to select one single day as sample day. A single day would not be fair to the disease bacteria; if we chose a spring day, we have very few typhoid cases, and on a summer day, there is little whooping cough and measles. Our original question is thus changed to the wording: How many bacteria are born each year in the United States?

Let us start with the bacteria of our intestine. They are necessary for life, hence we shall make our estimate low so that we can safely say that there must be certainly more than our estimate shows. Our intestine harbors many different kinds of bacteria: streptococci, lactobacilli, bacterioides, veillonellas, clostridia, and others, but all of these together are not as numerous as *Bacterium coli*. They all live on the food which we have eaten, and they move with it slowly through the intestine. Finally, when the food is excreted, they are all carried into the sewage. Of all these bacteria, *Bacterium coli* is most easily counted, and the number of coli bacteria in sewage has been determined many times. Each sewage plant knows how much sewage per person runs through the plant, and from these data, the number of coli bacteria per person per day can be computed. All such computations agree fairly well that the average person excretes about 250,000,000,000 coli bacteria per day. (This number can be written 25×10^{10}.)[1] With 120 million peo-

[1] To avoid the confusion caused by the many zeros, it is customary with astronomers and bacteriologists to give the number of zeros as exponent of 10. The above number could be written 25×1^{10} or 250×10^{9} or 2.5×10^{11}. Multiplication is made by adding the exponents. Thus, 3000×200 would be $3 \times 10^{3} \times 2 \times 10^{2} = 6 \times 10^{3+2} = 6 \times 10^{5} = 600{,}000$.

ple in the U.S., this makes a daily production of $120 \times 10^6 \times 25 \times 10^{10} = 3 \times 10^{19}$ coli bacteria per day. The total number per year is 365 times as large, or, in round numbers, 10^{22} bacteria (this is a 1 with 22 zeros).

This is the number of *Bacterium coli* born in one year. The other intestinal bacteria are also useful, certainly not harmful, but we shall not count them at all, and thus we can state safely that the total intestinal bacteria born in one year are certainly more than 10^{22}. All other mammals also carry these and similar intestinal bacteria, so do the birds, and even the lower vertebrates all harbor large numbers of intestinal bacteria which are continuously excreted, and are continuously growing again in the intestine. It is certainly an understatement if we assume that the intestinal bacteria of all animals are as numerous as those of the human species. But as we want a low estimate, let us agree that the total number of intestinal bacteria in man is 10^{22} and in animals also 10^{22}.

Another group of harmless, useful or necessary bacteria are the soil organisms. Top soil of cultivated farm or pasture land contains 3 to 10 million bacteria per gram, and from 6 to 12 inches down, it averages still more than a million. This means 50 to 160 million bacteria per cubic inch of top soil, and more than 16 million for the lower half of the top foot. In order to make the estimate low, we shall choose here 50 and 15 million as the respective average numbers per cubic inch. As an acre has 6,272,640 square inches, the bacteria of the top six inches of an acre amount to $6 \times 6{,}272{,}640 \times 50 \times 10^6 = 1880 \times 10^{12}$ bacteria. In the lower six inches, the corresponding number is 565×10^{12}, which makes a total of $2{,}445 \times 10^{12}$ bacteria per acre-foot. There are still several hundred thousand bacteria per gram of soil below the depth of one foot, decreasing rapidly as we dig further down, but we shall neglect them for good measure.

In forest soil, the number is smaller, and in order to keep

our average low, we shall consider 1 million per gram for the top soil, and 300,000 for the lower half of the top foot. This means 15 million and 5 million bacteria per cubic inch, and a total of 742×10^{12} bacteria per acre foot of forest land.

The United States contains about 3 million square miles, distributed in the following way:

320,000,000 acres of crop land
268,000,000 acres of pasture land
462,000,000 acres of forests and woodland
346,000,000 acres of poorer farmland not included above
153,000,000 acres of idle, failure and waste land, deserts, swamps.
61,000,000 acres of towns, cities, roads, railroads
263,000,000 acres of grazing land
1,873,000,000 acres total = 2,920,000 square miles

The first two items represent the best soil with the highest bacterial count. These 588 million acres contain each $2{,}445 \times 10^{12}$ bacteria, a total of 1.44×10^{24} bacteria. The 462 million acres of forest land harbor each 742×10^{12} bacteria, totalling 0.34×10^{24} bacteria. Thus, the best soils of the States contain together 1.78×10^{24} bacteria.

This estimate has been made very low, and it includes only about 1,000 million acres of land. The remaining 823 million acres cannot be estimated in regard to their bacterial content because they are very different. Certainly, they still contain an enormous number of bacteria, but they shall be considered here only in so far as to round off our estimate from 1.78×10^{24} to 2×10^{24} bacteria.

However, we cannot disregard that this represents the count on one single day. Soil bacteria do not multiply daily in large numbers as the intestinal bacteria do, but they do multiply occasionally. The number of soil bacteria goes up rapidly after plowing, still more after manuring or green manuring. These newly born bacteria die again

slowly, and finally, the soil has reached its normal numbers again. There is further a continuous slow change brought about by drought and rain, by freezing and thawing, which cause death and new growth. It is a very safe guess to assume that the average soil bacterium dies and is replaced about 5 times in the course of a year. This brings the number of soil bacteria born in one year to 10×10^{24}, as a very low estimate.

It is not very generally known that large numbers of bacteria live on the surface of plants. Leaves and stems contain between 10,000 and more than a million bacteria per gram of fresh material. The bacteria multiply during dew and after rain, and dry up in sunshine without losing their vitality. To compute their number in the United States, we must know the total weight of plants grown in one year, and also the average number of bacteria per plant.

The average number fluctuates greatly. Besides, a good many bacteria will be washed down onto the ground by heavy rains, and others will grow in their place. The ultraviolet rays of the run will probably kill those directly exposed, and they are replaced by new growth. The total number born in one year is certainly much greater than those counted on one single day. We have no good experimental data about this replacement, and in order to remain conservative, the average of 100,000 bacteria per gram is considered here to include all multiplication during the year.

It seems just as difficult to estimate the total plant growth of one year. The data on the individual crop plants are not complete, and there is a very large area covered by plants which are not included in any official estimate. We shall approach the problem from another angle. The total area on which plants grow normally can be computed from the survey reported two pages earlier as 1,660,000,000 acres. The average plant growth per acre can be estimated quite conservatively as one ton. Hay usually gives a much larger crop, and that is already dry, and had weighed much more

when fresh. The estimate of 1.6×10^9 tons $= 1.6 \times 10^{15}$ grams is therefore very low, and as each gram averages 10^5 bacteria, the total bacterial growth in one year on the plants of the United States is certainly more than 1.6×10^{20} bacteria.

The number of bacteria in water must be small in comparison with the soil bacteria, because the United States has far more soil than water, and because soil, on the average, contains about 10,000 times as many bacteria per cubic centimeter as water. If we discount the sewage bacteria of human or animal origin which have already been counted as intestinal bacteria, we can consider the average number of bacteria in fresh water to be approximately 100 per cubic centimeter.

The estimate of the number of water bacteria is based on the amount of water discharged into the ocean. The twelve largest rivers of the United States discharge a little more than a million cubic feet per second into the oceans, or 32 trillion (32×10^{12}) cubic feet per year. This corresponds to 88×10^{16} cubic centimeters. With 100 bacteria per cubic centimeter, the twelve largest rivers dump about 88×10^{18} bacteria into the ocean.

All these bacteria were born somewhere in the rivers during the year, but the total number born in water must be considerably larger because many bacteria die before they land in the ocean: they are killed by sunlight, by frost, by starvation, or they are swallowed by protozoa. Besides, our figures represent only the drainage of the twelve largest rivers. All the smaller rivers are not included, nor the stagnant inland lakes and ponds without drainage. If we multiply the above figure by 10, and round it off to 9×10^{20} to make allowance for all that was not included above, our estimate is still very low.

Now we come to the estimation of the disease bacteria born in the United States in 1941. The U.S. Public Health Reports list the following numbers of cases in 1941:

Whooping cough	183,273 cases
Scarlet fever	155,707
Pneumonia	141,939
Tuberculosis	103,348
Dysentery	19,151
Diphtheria	16,252
Septic sorethroat	10,198
Typhoid and paratyphoid fever	9,658
Undulant fever	3,358
Tularemia	1,641
Meningitis	1,631

Diseases with less than 1,000 cases need not be included because in comparison with the above, their bacteria are a negligible quantity. In estimating the number of disease bacteria, we shall make the estimate so large that we are certain that the actual number is lower than our estimate.

In whooping cough, the bacteria are located on and between the cilia of the finer bronchi and bronchioles, more rarely in the alveoli. They produce an exudate which is coughed up. The amount of exudate has not been measured. The total exudate coughed up in one day will certainly be less than the total air space in the lungs which is about 3 liters. If we assume that a whooping cough patient produces 3 liters of exudate per day, that is certainly an overstatement. The number of bacteria in ordinary culture media never exceeds a trillion per liter, and therefore 3 trillion = 3×10^{12} bacteria per patient per day is a very high estimate. Recovery from whooping cough is slow. The Danish government for instance insists on four weeks of quarantine. The total number of bacteria per case is thus about $30 \times 3 \times 10^{12} = 10^{14}$ bacteria, in round numbers. As there were 183,273 cases in 1941, the total whooping cough bacteria born in 1941 must have been less than $180{,}000 \times 10^{14}$ or less than 18×10^{18} bacteria.

With pneumonia, the calculation is similar. An exudate fills the alveoli. Assuming that not only the alveoli, but the entire lungs were filled with exudate, containing a

trillion bacteria per liter, we would have 3×10^{12} bacteria per patient per day as in whooping cough. The assumption that this total volume with its bacteria is renewed every day is quite wrong, but as in some cases of pneumonia, the bacteria get into the blood where they can increase to larger numbers, we shall cover this by the assumption that 3×10^{12} are growing every day while the disease lasts. Pneumonia is a rapid disease, reaching the climax in 5 to 10 days. The total number of pneumococci in the average case cannot therefore be more than 3×10^{13}, and the 141,939 cases in 1941 produced less than $3 \times 150{,}000 \times 10^{13}$ or less than 5×10^{18} pneumococci.

The number of bacteria in scarlet fever seems to be much smaller. There is a formation of pseudo-membranes, but no great quantities of exudate. The same is true with diphtheria where the membranes, originating from a fibrinous exudate may become so thick as to interfere with breathing. It seems impossible that these membranes can contain more bacteria than the large quantities of exudate in whooping cough and pneumonia. Thus 3×10^{12} bacteria per patient per day is an exceedingly liberal assumption. Incidentally, this is 12 times as many bacteria as are growing daily in our intestinal tract, and it seems hardly probable that the relatively small infected areas of respiratory diseases could produce so many bacteria. It confirms our conviction that we are exaggerating the number of disease bacteria enormously. We might as well add the cases of septic sore throat to those of scarlet fever and diphtheria, and consider all of them to last 30 days, so that the total for these three diseases, with 182,157 cases, resulted in less than $182{,}000 \times 30 \times 3 \times 10^{12}$ = less than 18×10^{18} bacteria.

In typhoid fever and dysentery, the pathogenic bacteria are continuously eliminated with the normal intestinal bacteria, and continue to multiply similar to *Bacterium coli* discussed a few pages back. Thus, the longer the disease lasts, the greater will be the number of disease bacteria born.

It is known that some people never get rid completely of the disease bacteria, and excrete them in small numbers for the rest of their lives. These so-called carriers are rare exceptions. If we make the assumption that the disease bacteria are entirely taking the place of the colon bacteria, and multiply at the rate of 25×10^{10} per day, and that this number of bacteria is excreted for 30 days, this exaggeration will be very ample to more than compensate for all bacteria excreted by carriers. This would make the total population of typhoid, paratyphoid and dysentery bacteria born in one year in the United States (28,810 cases) amount to less than $28{,}810 \times 30 \times 25 \times 10^{10} = 22 \times 10^{16}$ bacteria. This is a very small number as compared with the bacteria from respiratory diseases.

Tuberculosis in cattle has been combatted so successfully that according to official tests, not more than one-half percent of the cow population of the United States is afflicted. One-half of one percent of the 74,000,000 cattle in 1941 means 375,000 tuberculous animals. Tuberculosis is a slowly developing disease, the bacteria do not multiply rapidly, and remain mostly in the place where they grew. A veterinary friend of mine estimated that the average number of tubercle bacteria in a cow could not possibly be more than a billion, but to make quite certain of an overstatement, let us assume 10 billion = 10^{10} bacteria per cow, and let us further assume that they die 10 times during the year and are replaced by new growth. That would make the total population of bovine tubercle bacteria for one year equal $10 \times 375{,}000 \times 10^{10} = 4 \times 10^{16}$ bacteria.

Human patients with tuberculosis are not likely to harbor more disease bacteria than cows. The 103,348 cases of human tuberculosis in 1941 would thus not result in more than 10^{16} bacteria. This makes the total of human and bovine tubercle bacteria 5×10^{16} bacteria.

Then, we should consider the cases of chicken and other tuberculous animals. No estimates can be given for the

frequency of tuberculosis among smaller domestic animals and wild animals. To be sure of an over-estimate, let us multiply the above number by 4. The result is 20×10^{16} which represents an overestimate of the tubercle bacteria on all animals in the United States.

All of the more frequent diseases have now been accounted for. Next in importance is undulant fever, 3,358 cases, caused by a small bacterium which grows mostly in the blood. The number of bacteria in the blood is never very large, but the disease lasts long, and probably the bacteria die frequently and are replaced frequently. If we make the high estimate of a billion bacteria in the blood, to be replaced once a day, and if we assume the illness to last through the entire year for all patients, the total number of bacteria is $365 \times 3{,}358 \times 10^9 = 12 \times 10^{14}$. This number is so small that it is negligible in comparison, although it is an enormous number in itself.

The total number of disease bacteria produced in one year by the main human epidemics adds up to less than 41 quintillion bacteria. There are other common bacterial diseases which are not dangerous, but nevertheless an impairment of health, causing colds, diarrhoeas, boils, sores, wound infections and so forth. It is not likely that their total number could approach that of the great epidemic diseases, but in order to continue our exaggeration, let us assume that there are so many of them that they bring the total of human pathogenic bacteria to 100 quintillion. Then there are the diseases of animals about which we have no statistics whatever. Let us assume their total to be twice that of human pathogens, or 200 quintillion. There remain still the disease bacteria of plants. Most plant diseases are due to molds, but no statistics exist concerning them or the plant-pathogenic bacteria. However, we can rely on our observation that far more plants are healthy than showing disease, and if they show disease, it is usually only on certain spots of the leaf or stem while the rest of the leaf or stem is healthy.

CENSUS OF THE BACTERIA IN THE UNITED STATES IN 1941

Bacteria in Soil	more than	10,000,000,000,000,000,000,000,000
Bacteria in Human Intestine	more than	10,000,000,000,000,000,000,000
Bacteria in Animal Intestine	more than	10,000,000,000,000,000,000,000
Bacteria in Inland Waters	more than	900,000,000,000,000,000,000
Bacteria on Plants	more than	160,000,000,000,000,000,000
Total harmless, useful or necessary bacteria	more than	10,021,060,000,000,000,000,000,000
Whooping Cough	less than	18,000,000,000,000,000,000
Pneumonia	less than	5,000,000,000,000,000,000
Scarlet fever, Diphtheria, Septic Sore Throat	less than	18,000,000,000,000,000,000
Bacteria of Typhoid, Paratyphoid, Dysentery	less than	220,000,000,000,000,000
Tubercle bacteria in man and all animals	less than	50,000,000,000,000,000
Undulant fever	less than	1,200,000,000,000,000
All other human diseases	less than	59,000,000,000,000,000,000
Total disease bacteria of man	less than	100,271,000,000,000,000,000
Total disease bacteria of animals	less than	200,000,000,000,000,000,000
Total disease bacteria of plants	less than	8,000,000,000,000,000,000

The number of disease bacteria on plants is probably less than one percent of the total number which we had estimated above to be more than 1.6×10^{20}; but we may assume 5% or 8 quintillion, in order to be on the safe side.

If we summarize all these data in two groups, good bacteria versus bad bacteria, we find more than 10,031,000 quintillion good ones against less than 308 quintillion bad ones. In other words, out of every 30,000 bacteria in the United States, 29,999 are harmless, useful or even necessary for our lives while one is a disease bacterium. That is not a bad record compared with that of the human race. In 1942, there were 7,569 persons convicted of murder in the United States, or 1 out of every 17,000. Considering that the proportion of harmful bacteria in our estimate is certainly too high, it would only be fair to admit that bacteria are certainly no more dangerous to humanity than man himself.

Obviously, the Lord did not create microbes to be a scourge to man, animals and plants. They are as necessary to our universe as all other plants and animals, and if once in a while, one out of many thousand is found to injure or even kill people, it should be remembered that few other organisms can show a better record.

Chapter Thirteen

HOW YEASTS WERE DOMESTICATED

As long as we can trace human history, man has made wine or similar alcoholic beverages. All African tribes had such drinks before they entered "history" by being discovered by the white man. Chinese rice wine is mentioned at 2200 B.C. Noah's vineyard is still on exhibit for gullible tourists. But man did not invent wine, nor does man make wine. It is the yeasts that do the work. And yeasts are not partial to man; they make alcohol for anybody who likes it, and many animals love alcohol. Alcohol is a great treat for insects. The collector of big night moths lures them by fermented fruit pulp. Wasps love alcohol. Fly traps can be baited with it. Psychologists have found that mice prefer 4% alcohol to plain water but they were not interested in stronger mixtures. The difference between men and animals is largely that men try to preserve their drinks so that they can store them, and also, their urge for "bigger and better" has taught them to concentrate their liquors.

To make wine, nothing is needed but fruit and a vessel. To be sure, by fruit is meant what the housewife calls fruit, namely apples, pears, grapes, oranges and the like, not what the botanist calls fruits, such as pumpkins, walnuts, string-beans and pine cones. In other words, it must be sour, and it must be sweet. If such real fruit is crushed, the pulp will soon begin to ferment. Yeast need not be added because it is already there. All fruits contain yeasts on their skins. Sound grapes from the famous New York wine district carry 1000 to 300,000 yeast cells per berry, and injured berries, picked by a bird or bitten by a wasp, may contain as many as 60 million cells. Grape juice directly from the press averages more than half a million cells per cubic centimeter, and the remaining pulp contains much more. If the temperature is

not exceedingly low, yeast multiplies readily in the crushed fruit and ferments the sugar to alcohol.

How so many yeast cells get onto the grapes, has been studied by the wine experiment stations of Europe. There are very few yeasts on the young green grapes, but in the soil of the vineyards, a fair amount survived from Fall to Spring. When the grapes begin to get ripe, insects fly from one berry

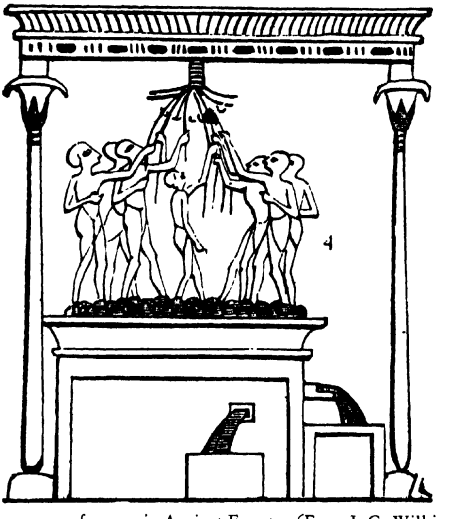


FIG. 59. The pressing of grapes in Ancient Egypt. (From J. G. Wilkinson: The ancient Egyptians.)

to the next, hoping to find one that is injured and exudes juice. The most active insects of the vineyards are the wasps and they do not have to wait for injured fruits, they can bite through the skin. All insects carry on their legs tiny particles of the soil to the grapes and the soil contains yeast. Wind also may blow dried soil with yeast cells around. In the wounds of the injured grapes, yeast multiplies rapidly, and produces alcohol. The smell of alcohol attracts flies and wasps, they feast on the fermenting juice and get thousands of yeast cells on their legs which are then carried to

other berries. Thus, during the few weeks of ripening, yeasts are being spread rapidly throughout the vineyard.

The importance of wasps for the inoculation of grapes with yeast became evident in a very cool and wet year when the grapes of the Rhine valley developed tough skins through which the wasps could not bite. When the grape juice was pressed, it did not ferment promptly as in former years, but remained dead for many days, and undesirable changes began to set in. Finally, the few yeasts had multiplied sufficiently to start the fermentation, but the damage to the flavor of the wine had been already done.

When fruit pulp ferments, the pulp contracts and settles to the bottom. After the violent, frothy fermentation is over, the yeast too settles out, leaving a clear liquid, the wine. This wine, with its acid and alcohol content, is not a good medium for most bacteria and yeasts; only one kind prefers wine to any other food, namely the vinegar bacteria. They grow on the surface of the wine and oxidize the alcohol to acetic acid, the acid of vinegar. There is one simple way of keeping the wine from turning to vinegar, and that is to keep the air out. Without air, the alcohol cannot be oxidized, and the vinegar bacteria cannot multiply. The ancient amphoras of Greeks and Romans with their very narrow necks appear very unpractical until one learns that they took the place of our wine kegs. They were filled to the neck with wine, and then, olive oil was poured into the neck which prevented the air from coming in contact with the wine. For transportation over long distances, they were sealed with pitch. This preserved the wine very well, but it seems unavoidable that quite a little oil got into the wine goblets, at least at the start of a drinking bout. Some scoffers say that was the reason why these feasts always started with a libation to the Gods by pouring a little wine from the first goblet on the earth. The Gods got the wine with the oil.

Yeast ferments sugar to almost equal amounts of alcohol

and carbon dioxide. The latter, being a gas, escapes, and the wine contains approximately half as much alcohol as there was sugar in the fruit juice. Grape juice contains between 18 and 24% sugar, and grape wine thus will contain between 9 and 12% alcohol. Sometimes, in a cool and wet season, a little sugar is added to grape juice to bring the alcohol content above 10% as it may not keep so well with less alcohol. Cider has not more than 12% sugar usually, and therefore hard cider contains only 6% alcohol unless sugar had been added.

Most wine yeasts cannot produce more than 12% alcohol, and if grapes have more than 24% sugar, the excess remains unfermented and sweet wine is obtained. The subtropical climates produce certain grapes with such a high sugar content. In Spain and Algiers, certain sweet wines are made by drying the grapes for a few days before pressing, to get an extra sweet grape juice.

The quality of wines varies greatly if one can believe that the price paid for some rare vintages is a real measure of quality. Many experiments have been made to improve low grade wines by fermenting them with pure culture yeasts from high grade wines, but improvement was so insignificant that the idea has been given up altogether. The quality depends primarily upon the grape variety, then upon the soil, and to a great extent upon the weather. A warm dry autumn brings forth not only a high sugar content, but a delicate aroma of the grapes which is imparted to the wine.

An interesting saga is told about the discovery of a wine improvement by a special mold growing on the grapes. Some centuries ago, the Bishop of Mayence was very ill. To his bishopric belonged large vineyards which were closed as soon as the grapes began to ripen, and to which nobody was admitted until the bishop gave the signal for the grape harvest. But this year, when the grapes were ripe, the bishop was too ill to give the signal, and the grapes became moldy. A gray powder spread gradually over the grapes

which turned brown and began to dry up. Not until November did the bishop recover sufficiently to give the signal for the harvest. It seemed hardly worth while to gather these ugly-looking grapes, but never before had such a high quality wine been obtained from these vinyards. In the Rhine valley, but also in other regions, e.g. in the

FIG. 60. Grape pressing at Urbana Wine Company, Hammondsport, N.Y. The grapes, after being stemmed and crushed on the upper floor, are spread about 4 inches deep on layers of cloth. Large stacks of these layers are then subjected to slowly increasing hydraulic pressure (left). (Photo: Mrs. June Alexander, Bath, N.Y.)

Hungarian provinces of the Tokay wine, this special mold is greatly desired. It develops only under very specialized climatic conditions. Its name is *Botrytis edulis*.

In American wineries, the grapes for white wine are pressed in large hydraulic presses. They are spread in a thin layer on a heavy cloth which rests on a wooden frame. Twenty or more such frames with grapes are stacked one over the other, and then pressure is applied and very gradu-

ally increased. For better quality wines, the grapes are sorted before pressing, and green and moldy berries are removed. The colorless, cloudy juice runs into a tank or large cask. In some wineries, it is inoculated with a pure culture wine yeast. As a rule, the fresh juice contains nearly a million yeast cells per cubic centimeter, and will begin to show the first bubbles of fermentation in less than 24 hours if the weather is still warm.

In another day or two, fermentation becomes quite violent, a thick froth covers the liquid, and its temperature rises 10 or more degrees. The winemaker has to watch this carefully. Fermentation may become so violent that the temperature rises over 100°F., and the yeast is killed. It has committed suicide, so to speak. That is likely to cause a poor grade of wine. In California, artificial refrigeration must sometimes be applied to prevent this. Normally, in a few more days, fermentation calms down, bubbling ceases and the young wine which was very turbid from the yeast growth, slowly clears because the yeast settles to the bottom.

After the wine is fairly clear, it is racked, i.e. it is syphoned or pumped to another cask, leaving the sediment, the lees, behind. The wine must now be carefully watched to keep the vinegar bacteria out. This is accomplished by careful cleaning of the casks and by fumigating them with burning sulfur before filling. The sulfur fumes are an ideal disinfectant and are used in all yeast industries because they do not injure the yeast very much, but kill the vinegar bacteria and many other species. As a further precaution, all casks are kept absolutely full to the bunghole. Very little air can get into a cask, with several thousand gallons of wine, through a bunghole of perhaps two inches. Without air, vinegar bacteria can do no harm.

Red or blue grapes when pressed in this way give a white grape juice and a white wine. The customary red grape juice is obtained by steaming the Concord grapes and pressing them hot. If a red wine is wanted, the grapes are not

pressed, but ground, and dumped into a tank in which they are allowed to ferment for a few days. The gas of fermentation will force the pulp to the top of the tank, and then, the young red wine can be drawn off from the bottom and pumped into a new cask where it is treated like the white wine.

Wine is not considered fit to drink until it has aged for about two years. During this ageing, it is kept cool and quiet except for occasional racking to make it clear. It is during this ageing period that the high bouquet develops.

The wine yeasts can hardly be called domesticated. They are naturally present in the vineyards, and most attempts of introducing pure cultures have been unsuccessful. Only in one famous wine product is a cultivated yeast in pure culture introduced, namely in the champagne manufacture.

Champagne is made from a finished wine, usually from a select, perfect wine with a special bouquet, by adding sugar and a pure culture of champagne yeast. This mixture is bottled at once, and placed in cool cellars where this special yeast which can tolerate more alcohol than the normal wine yeasts begins to multiply very slowly, and leisurely ferments the small amount of sugar. Gradually the carbon dioxide released by the yeast produces a pressure of about 90 pounds per square inch, and it is not unusual to see in the racks of these bottles some that have been blown to bits by this pressure. The flavor of the champagne requires between one and two years for full development. Then, the bottles are placed on special racks with their necks down, and are shaken slightly every day for a few weeks until finally all yeast has settled on the stopper. The neck is then dipped into a freezing mixture, and when the yeast is frozen solid, the cork is removed, the inside pressure forces the yeast out, the bottle is filled up, a new cork is put in, and the champagne has lost but little of its carbon dioxide charge. It is sparkling clear now, and ready for sale.

The wine fermentation is a natural fermentation, taking place without inoculation. The manufacture of all other

alcoholic beverages is not natural, but enforced. If it were not for the interference of man, the materials used to make beer or whiskey would not undergo yeast fermentation, but would sour. In the fruit juices, no bacterial growth can take place because of the acidity, and yeast is always present. In the other alcohol industries, the raw materials are not

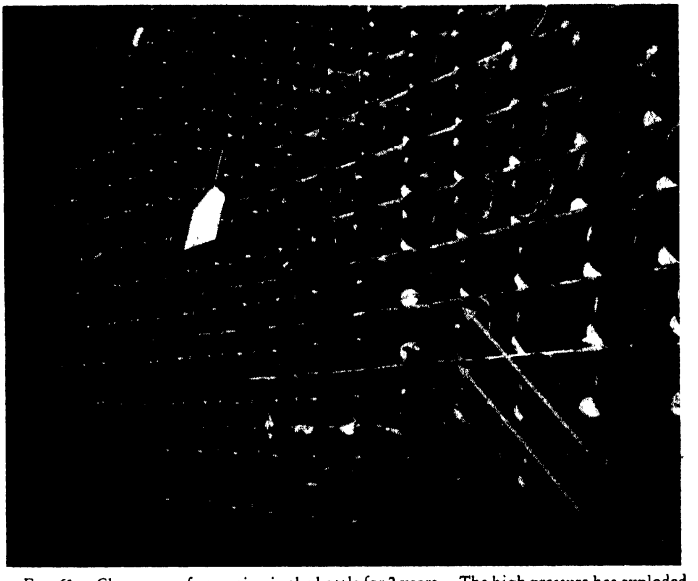


FIG. 61. Champagne fermenting in the bottle for 2 years. The high pressure has exploded two bottles. (Urbana Wine Company, Hammondsport, N.Y. Photo: Mrs. June Alexander.)

acid, and yeast must be added in very large amounts to crowd out all other microorganisms, and in most cases, the medium is also sterilized by heat.

The Brewing Process. This is especially true for the beer fermentation. Beer manufacture is an old industry; the ancient Egyptians were expert brewmasters; according to Herodotus, they had learned it from their goddess Isis, wife of Osyris. Tacitus (57 A.D.) claims that the Germans learned this art from the Egyptians. With us, it has developed into a great industry.

The raw material for beer is barley, with eventual addition of other grains, but barley is the backbone of it. The official definition is "malt beverage." Barley consists largely of starch, and yeast cannot ferment starch. When

FIG. 62. The change of barley to malt by two different varieties of barley. (Reproduced with permission from the American Society of Brewing Chemists' Proceedings. Courtesy of Wallerstein Laboratories, New York.)

barley germinates, the starch is slowly changed to maltsugar, and so, beer is made from germinated barley which is called malt. The malting requires much skill and experience, and is not done in the brewery any more, but in a special malt house. Malting is now a separate industry, and the brewer buys his malt. The maltster must know his barley because

only few of the many barley varieties give a good malt for brewing.

The barley is steeped for 2 or 3 days in water, then spread on the malt floor in a layer one or two feet deep where the wet grains slowly begin to germinate. The temperature must be carefully watched, because germination is accompanied by an intense respiration which produces so much heat that the grains have to be turned over frequently, with a shovel, or eventually in revolving drums, to cool off, and also to get more air for respiration. After about ten days, the sprouting has gone far enough. The barley has rootlets several times the length of the kernel and the first green cotyledon leaf has pushed out of the seed till it is as long as the kernel. At this "green malt" stage, the germination process is interrupted by drying and kilning. This kilning or roasting is carried out at temperatures from 185 to 230°F. At the higher temperature, the malt becomes dark brown and yields a dark beer. This dry malt keeps. It still contains mostly starch, but in the sprouting process, the starch-destroying enzymes have been formed, and when the malt is now ground and mashed with warm water, the enzymes change all the starch in a few hours to malt sugar. This mashing is one of the most important phases in brewing. Upon its successful operation depends the quality of the beer. Several different procedures are used, differing in the times and temperatures. If corn starch or rice are added at this stage, their starch is also converted to maltose. When all starch has been changed to sugar, the brewer boils the mash, filters it, boils it again with hops, filters it again, cools it thoroughly, and adds his pure culture yeast.

This malt extract, called the wort, contains no starch, plenty of sugar, some dextrines which are compounds halfway between starch and sugar, most of the protein of the barley, and the bitter hop oil. This hop oil is not there just for the flavor. It is an antiseptic which does not affect the yeast, but is quite toxic to lactic acid bacteria. If it

were not for the hops, the wort would be an ideal medium for bacteria, and they would make the entire wort acid in a very short time. Some types of beer are made without hops, notably the Berliner Weissbier and the Lichtenhainer in Germany. Both contain lactic acid bacteria in large quantities, and taste decidedly but pleasantly acid.

FIG. 63. A hop vine, with the hop fruits which are used in brewing. (Courtesy of Brooklyn Botanical Gardens.)

A very large amount of yeast is added to the wort, and in the nutritious malt extract, it multiplies rapidly though the temperature is held very low, about 45°F. for lager beer and 60° for ale. Strangely, the brewers in America use neither the Fahrenheit nor the Centigrade scale, but they retained the old Réaumur scale. In the warmer ale vats, the first bubbles appear very soon, and after two days, the entire vat is covered with a thick layer of froth. The fermentation often gets so wild that the foam runs out of the vat. The

lagerbeer fermentation is slower and lasts 8 to 10 days while ale is finished in 5 to 7 days. Then, the foam collapses, the yeast settles and leaves a slightly turbid beer.

This fresh beer does not taste good. It is drawn into a large storage cask where it is kept for perhaps two months. Lager beer is the German word for stored or aged beer. It

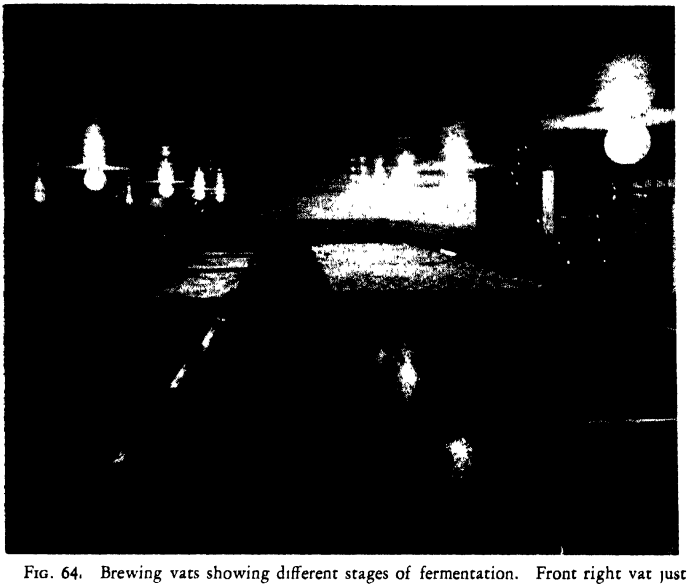


FIG. 64. Brewing vats showing different stages of fermentation. Front right vat just starting, back right vats with heavy foam, left vats nearly finished. The vats are very deep, holding 10,000 gallons each. (Courtesy of Piel Brothers, Brooklyn, N.Y.)

develops its full flavor slowly, and the last of the yeast settles out during storage, making the beer perfectly clear.

Pasteur wrote a book about beer fermentation and improved French brewing by correcting some of its worst troubles, at a time when pure cultures were unknown. The first pure cultures were introduced into practical brewing by Emil Christian Hansen of the Carlsberg Brewery in Copenhagen in 1881. He began his important work by developing

FIG. 65. Storage tanks for the ageing of beer. Each tank holds 25,000 gallons. (Courtesy of Piel Brothers, Brooklyn, N.Y.)

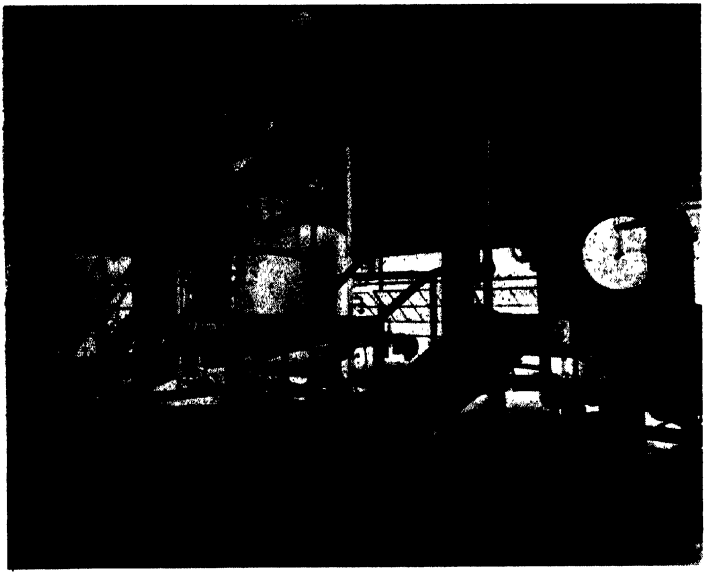
a method which gave him a pure culture from a single yeast cell. After he had tried out these cultures in his brewery, he finally succeeded in convincing the entire industry: of the advisability of pure culture yeasts, of the necessity of sterilization and antisepsis and utmost cleanliness, and of

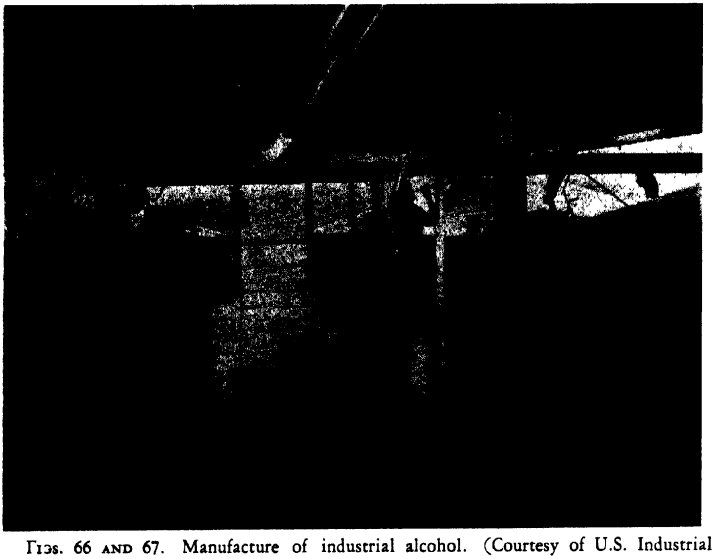
the advantages of microscopical as well as chemical control of the entire brewing process. Through his ceaseless efforts, the brewing industry was far ahead of all similar industries. When the dairy industry, 15 years later, began its first attempts at pasteurization of milk, and at pure culture cream ripening, it found the trail blazed by the breweries.

Industrial Alcohol: Distilled alcohol is manufactured either from the cheapest source of sugar, which is molasses, the residue of the sugar manufacture; or from some inexpensive starch, such as corn or rye or sweet potatoes in this country, sugar beets in France, potatoes in Germany and sulfite liquor from the wood pulp industry in Sweden. The simplest process is the manufacture of industrial alcohol from molasses. The so-called black strap molasses, the cheapest product available, must first be treated chemically to make it suitable for yeast growth. Then it is diluted to about 12% sugar, and its acidity is adjusted to suit the yeast. As molasses contains very little protein matter, and as the yeast must grow and multiply in it, some ammonium phosphate is added to give the yeast nitrogenous matter for growth.

To this dilute molasses, the pure culture yeast is added, about one-twentieth of the total amount to be fermented. That is quite a large amount of pure culture because the fermenting vats often contain more than 100,000 gallons, which means a requirement of about 5,000 gallons of seed yeast. Every pure culture starts with a testtube culture. This is poured into a pint flask of malt extract. When this begins to ferment, it goes into a gallon of malt extract. As soon as this bubbles, it is used to inoculate a 10 gallon tank, and this is then poured into the final batch of 300 gallons of sterilized molasses from which the big fermenters are seeded.

The seeding is heavy, all conditions (temperature, acidity, yeast food) are made ideal for yeast growth, and the fermentation is completed in about 50 hours. By this time, all sugar has been changed to alcohol, and the mash is now





FIGS. 66 AND 67. Manufacture of industrial alcohol. (Courtesy of U.S. Industrial Chemicals, Inc.)

Above: A 300 gallon tank with sterilized molasses is being inoculated with a pure culture of yeast cultivated in a glass flask.

Below: In these large tanks, each of 134,000 gallons capacity, of which only the top is visible, the final fermentation of molasses is carried out by the yeast grown in the above 300 gallon tank.

heated. It contains only 6–8% alcohol, but as alcohol boils at a lower temperature than water, it distills off first. A distillate of about 60% alcohol is obtained, which is redistilled to produce the usual industrial alcohol of 95%.

As has been explained above, only one-half of the sugar can be changed to alcohol while the other half goes into carbon dioxide gas. In large distilleries, and even in some breweries, this gas is collected and compressed and sold for various purposes. Our picture shows that the tops of the fermenters are closed, and have wide pipes conducting the carbon dioxide to the compressors.

Industrial alcohol is also made from grains. This is usually considered the purer product and commands a slightly higher price. As the yeast cannot ferment the starch of the grains, it must first be changed to sugar, and that is usually done by addition of barley malt. Rye malt or wheat malt, and even rice malt is sometimes used. The grain, mostly corn or rye, is heated with steam under pressure to make the starch easily miscible with water. This starch paste is mixed with an appropriate amount of malt, and kept at a temperature near 110°F. which is the best temperature for converting starch to sugar. When this is accomplished, pure yeast is added, and all further fermentation and distillation proceeds essentially as with molasses.

However, one trouble frequently arises with grains. While the malted mash is kept warm, the spores of butyric acid bacteria, of *Clostridium butyricum*, which are common in soil and on grains, and which survive heating, may germinate, and as these bacteria can multiply extremely rapidly, doubling eventually in 10 minutes, they may start a butyric acid fermentation which continues after the yeast has been added. The alcohol distilled from such a mash contains so much butyl alcohol and other obnoxiously smelling compounds that it is useless for most purposes. Rectification of such alcohol is quite difficult.

A rather simple remedy is used which was discovered by the German potato distilleries; they suffered even more than grain distilleries from butyric acid. The mash is heavily inoculated with *Lactobacillus Delbruecki*, a long, slender rod which grows well at 110°, and makes lactic acid from sugar. In such an acid medium, the Clostridium spores do not germinate while the yeast thrives in presence of lactic acid.

Whiskey Manufacture: The different kinds of whiskey are made by a process which is in no essential way different from the above description of industrial alcohol manufacture. More attention is paid to the quality of the grains used, rye for the rye whiskey and corn for the Bourbon whiskey. Special care is taken in the rectification to make the removal of all offensively tasting compounds as complete as possible, and the alcohol is distilled only to a concentration of about 60%. With all precaution of rectification, the freshly distilled product tastes so raw as to be undrinkable. The law requires that all whiskeys must be aged for at least two years in charred oak barrels before being sold. During this ageing, a chemical change takes place which brings out certain desired flavors from the raw-tasting compounds of the fresh product. This change has nothing to do with yeasts or bacteria. It is characteristic of all alcoholic beverages, wine, beer, whiskey and brandy.

Brandies are distilled liquors made from fruits. Some of the fruit flavors are carried over in the distillation process which give the special aroma to cognac (grape brandy), apple jack, peach brandy and the like. Being distilled products, they are colorless, for the color of the fruit does not distill over. A slight brownish hue may be observed because brandies also have to be aged in charred oak barrels, and the alcohol may dissolve some coloring matter of the charred wood.

Malt Substitutes: The malt needed for the fermentation of grains is expensive, and other ways have been proposed to change the starch to sugar. One way is the boiling of starch with acid under pressure. Most interesting is the so-called Amylo-process which is the original Japanese method of making saké, the rice brandy. The grain is first heated, usually under pressure, to liquefy the starch. Then, the spores of a certain mold are sown into this mash which germinate and grow rapidly at about 100°F., and change the starch to sugar. They produce an enzyme which is essentially like that contained in the malt. The mold species commonly used belong either to the *Mucor* family, as *Mucor Rouxii* and *Rhizopus japonicus*, or to the *Aspergilli*, as *Aspergillus oryzae*. The mash is aerated for 24 hours, and then the yeast is added for the final fermentation.

A combination of the mold and malt process has been introduced recently by the manufacture of "moldy bran." This is an enzyme preparation made with the help of some molds which is cheaper than malt (see Chapter Nineteen).

Bread Yeast Manufacture: In all previous fermentations, yeast was merely used as a tool to accomplish a certain purpose, namely the manufacture of alcohol. When the yeast had accomplished its purpose, it became a waste product and it was customary to dump it in the sewer. More recently its high protein content has been realized and it is dried for livestock feed. Quite the opposite attitude is found in the bread yeast industry where the yeast is the desired product, and alcohol is a byproduct which is not always rescued, but may be dumped in the sewer.

This seems a great waste of material and energy, but it has its good reason. Beer yeast is not fit for bread yeast; it does not produce enough gas, and above all does not produce it quickly enough. Also, it contains the bitter hop oil which would spoil the taste of the bread. Distillery yeast is well fit for bread yeast, in fact, some yeast varieties serve as distillery yeast and also as bread yeast, but the distillery

yeast cannot be separated from the remains of the fermented grains which do not belong in the bread, and usually, the yeast is heated with the entire mash in distillation, and thereby killed.

To get the largest yeast crop from sugar, the yeast must

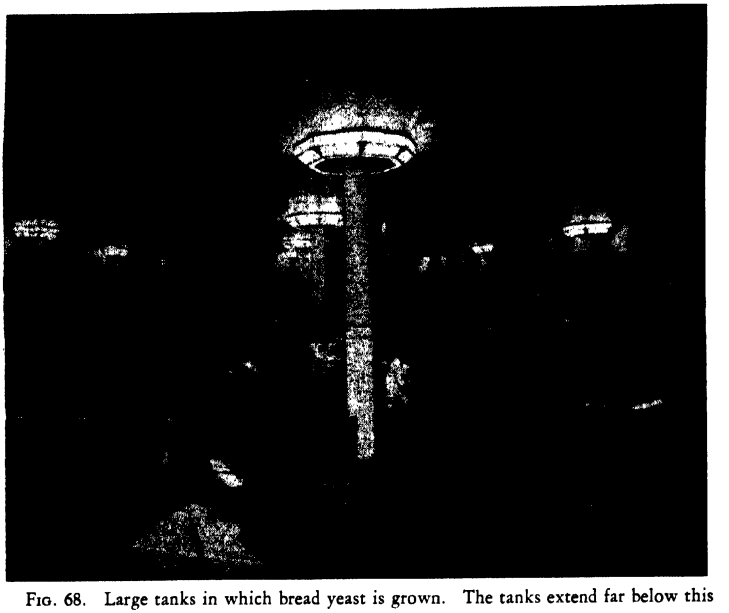


FIG. 68. Large tanks in which bread yeast is grown. The tanks extend far below this floor. (Courtesy of Fleischmann Laboratories, N.Y.)

not only get plenty of nitrogenous food, but must also be aerated. With a large amount of air, yeast makes only a little alcohol, but burns up the sugar completely by a respiratory process very similar to that of animals, and thereby gets much more energy from the sugar which documents itself in much more growth.

The cheapest source of sugar is molasses, but some yeast factories prefer the use of grains with malt. Molasses contains so little nitrogenous matter that some other source of nitrogen must be added to obtain good growth. A large part of this can be given in the form of ammonium salts

which the yeast can use well. Ammonium phosphate is frequently used because the yeast needs phosphate also for growth. Usually some organic nitrogen is also given, for yeast seems to produce a larger crop when part of the nitrogenous food is organic in nature. Malt extract, or acid peanut extract, are some of the common sources.

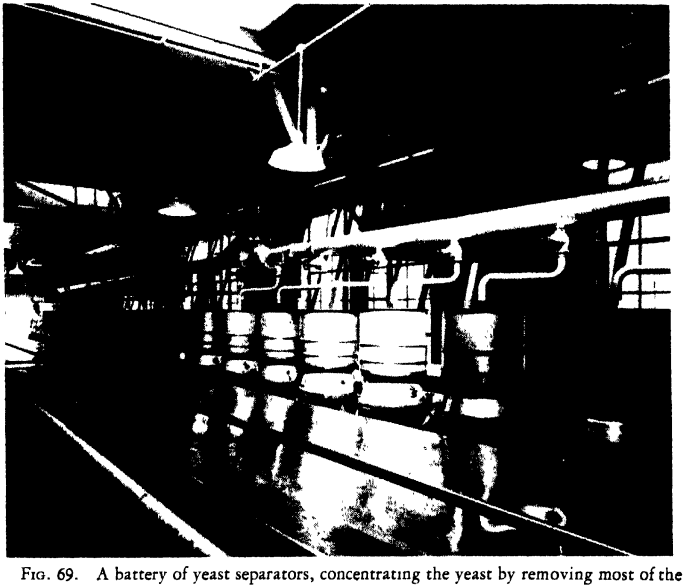


FIG. 69. A battery of yeast separators, concentrating the yeast by removing most of the wort. (Courtesy of Fleischmann Laboratories, N.Y.)

The yeast is grown in tall tanks, and the manufacture proceeds in the following fashion: A heavy amount of seed yeast is used, perhaps 300 pounds of pure culture yeast, which is stirred into 20,000 gallons of "wort." This wort is made from a very dilute molasses containing only about one-half percent of sugar, and a little ammonium salt. Aeration begins at once through perforated pipes at the bottom of the tank, at the rate of about 500 cubic feet per minute. The tank is only half full, but after two hours about 800 gallons of a stronger sugar solution are added, and also more ammonia.

From now on, more sugar solution flows into the tank slowly, either dilute molasses or grain mash, and also a corresponding amount of ammonium salt, until after 8 to 10 hours, the tank holds some 30,000 gallons. Aeration had been increased, when more sugar came into the tank, to about 1,000 cubic feet per minute. During all this time, the

FIG. 70. From the separators, the thick yeast suspension runs into filter presses. (Courtesy of Fleischmann Laboratories, N.Y.)

acidity of the mash, its temperature, and its ammonia content are carefully watched and adjusted when necessary.

Then, after 10 to 12 hours, aeration is decreased, and when all sugar and ammonium salt is used up, the contents of the tank are concentrated in yeast separators. The yeast is obtained as a creamy liquid which is run into filter presses, and pressed dry. It is then cut into pound cakes, or into smaller units, and is kept refrigerated until sold.

The yeast thus produced is tested for its baking qualities. This is done either by measuring how rapidly the yeast will

liberate gas from a sugar solution, and how much gas is made by 10 grams of yeast in two hours. Or a dough is made according to a standard recipe, and the time is measured that this dough needs to fill a standard baking form.

The various yeast industries are an essential part of the industrial development of the United States. In the last

FIG. 71. The compressed yeast from the filter press coasts down to the packaging room. (Courtesy of Fleischmann Laboratories, N.Y.)

normal year before the war, in 1939, U.S. breweries produced 1,670 million gallons of beer, U.S. wineries manufactured 232 million gallons of wine, and 835,000 gallons of champagne, the total output of distilled liquors amounted to 145 million gallons, and of industrial alcohol to 201 million gallons. No estimate can be made of the bread yeast industry. All told, that is quite a good year's work for a tiny, microscopic plant like the yeast.

Chapter Fourteen

WHEN BACTERIA BLUNDER

Long before man appeared on earth, bacteria were busy decomposing all dead matter and thereby maintaining the cycles of carbon, nitrogen and other elements. When man appeared, and began to store food from one day to the next, or from summer to winter, bacteria did not change their habits; they continued to decompose all dead organic matter. It is not possible to teach them to stay away from our food. Bacteria have no brains, they can not learn, and so a contest between the two parties began, one trying to preserve, the other trying to decompose. This contest went on for many thousand years. Man made only very slow gains, and the contest was not really won by man until the work of Pasteur became generally known, although the time-honored method of trial and error had taught the preservation of certain foods even to prehistoric people. The dry storing of grains, the salting of fish, the manufacture of cheese to preserve the most valuable parts of milk, the smoking and drying of meat by the American Indians are only few of the many examples of ancient, empirical food preservation. Even canning on a commercial scale was practised several decades before Pasteur. But often, these methods failed, some unusual spoilage occurred, and then, the people were utterly helpless.

In Padua, in 1819, a peasant found on his porridge red spots resembling blood drops. Next day, it happened again, and the scared peasant asked the priest's help. However, the blessings of the priest did not bring relief, the trouble spread, and the population became so terrified that a commission consisting of police officials, health officers and professors of the University of Padua, was appointed by the government to investigate this matter. One of the professors recognized the spots as being caused by a fungus. This

fungus could be transferred to other starchy materials, which subsequently became red. He gave it the name *Serratia marcescens* which is still accepted by modern bacteriologists. It is one of the pigment-producing bacteria.

This was neither the first nor the last appearance of the "miracle bacillus" which has been named by others *Bacterium prodigiosum*. Its earliest record in history was in 332 B.C. when the Macedonians under Alexander the Great besieged the city of Tyre. Bloody spots had appeared inside the bread, and the soldiers became scared and were ready to desert. Alexander called his most reliable soothsayer to interpret this phenomenon, and this clever politician explained the matter very convincingly. The blood spots were *inside* the loafs, so they meant bloody destruction for the people *inside* the walls. Nobody could find fault with such a convincing interpretation. The soldiers continued their siege and finally conquered Tyre.

In later centuries, red spots appeared occasionally on the holy bread of communion which was variously explained as a miracle, or as an offense to the Christian Church by the Jews, and resulted accordingly sometimes in the building of a chapel in memory of the miracle, and at other times in persecution and killing of the Jews. One commentator believes that this bacterium has caused the death of more people than some of the pathogenic bacteria.

Another simple example of unusual spoilage is the appearance of blue milk. In the good old times, about a century ago, life was crowded with more work than pleasure, and life was rich and worth living, for bacteria as well as for man. This was the heyday of *Bacterium cyanogenum*. The dairy maids of the old Holstein estate had a long day's work to milk all the cows, to bring the milk to the dark, cool milk cellars, and to pour it into the shallow dishes standing in long rows for creaming. Then, the cream which had risen on yesterday's milk had to be scooped off with a tin ladle, and collected in the cream vat. When the cream was sour

enough, it had to be churned, the butter had to be washed and salted and kneaded into crocks. The old skim milk was brought to the pigs, and then, the creaming dishes had to be washed and scrubbed and dried in the sun, because cleanliness was then as well as now the main virtue of a creamery. The price of butter at the fastidious Hamburg market was sure to drop if the dairy maids became careless about their dishes.

FIG. 72. A Holstein Dairy of a century ago. The dairy maid scoops the cream from the shallow pans into the cream vat. (From W. Fleischmann. Das Molkereiwesen, 1876.)

And then, one day, it happened. The cream was grey, and the next day, it had developed blue spots, as if blue paint had been splashed over the surface. When the mistress learned of the blue cream, her heart sank. She had heard enough about this scourge to realize that her dairy business was ruined. Blue milk gave a bad taste to butter, and once it came to a dairy, it remained for months or even for years. One of her neighbors had had it for eleven years. Some people claimed to have gotten rid of it by burning sulfur in the cellars while others had treated the cows with herbs

and drugs, but no reliable procedure was known to cure this trouble.

Similar stories can be told about gassy cheese, ropy bread, mushy pickles, moldy marmalade, pink sauerkraut, fermenting honey, putrid salt pork, poisonous sausages, and so forth. Such spoilage happened frequently, and often continuously. The people were at a loss to explain the scourge, sometimes blaming it to witchcraft. It still happens occasionally, and sometimes we know the cause, and know how to cope with it. But at other times, the spoilage is something new, never studied before, and it cannot be remedied until it is thoroughly understood.

It is not sufficient to say that spoilage is caused by bacteria. Different bacteria cause very different types of spoilage, and their suppression requires very different steps. A rotten egg, a rotten apple, and a rotten cabbage smell and feel and taste differently. Sauerkraut may become flat, or pink, or gassy, or putrid; wine may become brown, or cloudy, or vinegary, or bitter. To prevent these various types of spoilage, the causative organisms and their life habits must be thoroughly understood.

Not all foods spoil readily. Some are kept without any protection whatever for several months, as for instance potatoes or carrots, beets, pumpkins, and onions. Others spoil in less than a day when not cooked or refrigerated, such as milk and meat. The difference in keeping quality does not surprise us because we are used to it, but it is nevertheless remarkable. And it has a good and simple reason. The foods that keep are still alive; those that spoil are dead.

Bacteria will decompose dead organic matter readily, but they do not as a rule attack living matter. To be sure, some have fallen into bad habits and do attack living matter; we call them disease bacteria or pathogens. There are animal pathogens and plant pathogens. However, they are exceptions with which we shall not deal here.

Potatoes are living; if they are planted, they grow. Cab-

bages and spinach are alive until they are cooked. They grow no more, but they are still respiring, and so are apples and oranges. If they are crushed or heated, they are dead, and then they deteriorate rapidly.

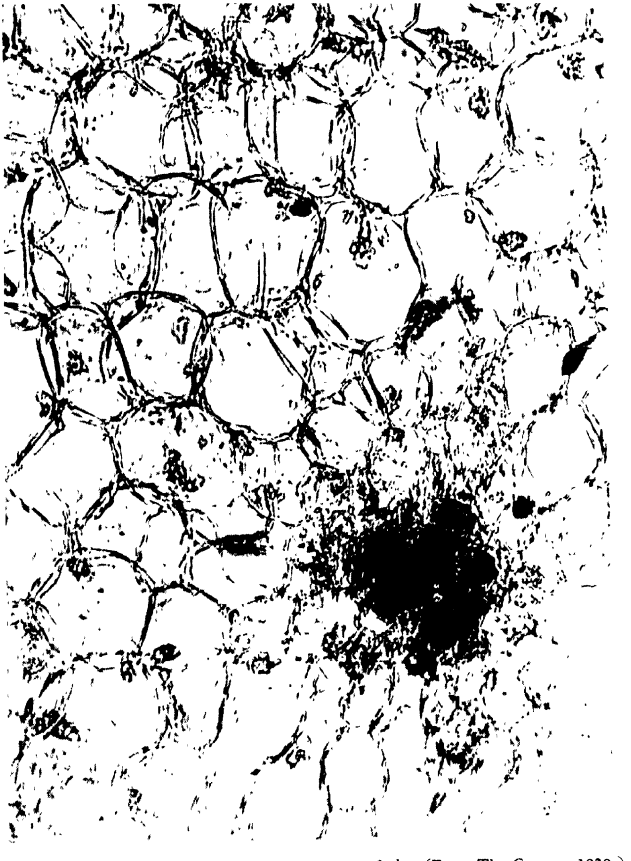


FIG. 73. The inside of a cucumber, 200X magnified. (From The Canner, 1929.)

All parts of any plant, roots, stems, leaves and fruits, are composed of thousands of tiny, microscopic units, called cells, which are glued together to a very solid structure by a gummy substance called pectin. Each plant cell is surrounded by a solid cellulose wall, and inside is the living protoplasm (see Figs. 73 and 74).

When a cucumber is cut from its stem, some cells are injured, bacteria can enter, and they begin to multiply, feeding on the rich cell contents. But they cannot penetrate from there into the uninjured cells, because of the solid cellulose walls. After the death of the cells, however, the cell wall offers no further protection. Boiled cabbage will not remain fit to eat for more than a day or two if kept exposed to bacteria at room temperature, and raw crushed cabbage starts to get sour promptly, and will turn to sauerkraut if treated right, or to a bad-smelling pulp if treated wrong.

Fruits do not keep as well as the sturdier vegetables. The cells of ripe fruits are old and have little vitality. Fruits are acid, and are not invaded by bacteria which do not like acid, but by yeasts and molds. Yeasts cannot spread from one cell to another, and thrive only in fruit juices, but the molds consist of long threads which have the power to puncture the cellulose walls and to grow from one cell to the next, destroying it and rotting in time the entire fruit. On a perfect apple or orange, they cannot gain a foothold. Yeasts and mold spores are always found on the outside of fruit, but they cannot develop because they have no moisture. If an apple is bruised, or lies on the damp soil, the mold spores can germinate and their hyphae will soon break through the skin and start spoilage. That is the reason why windfall apples do not keep, and why only hand-picked fruit is shipped over long distances. Oranges are wrapped to prevent spreading of mold spores. Even a cut orange cannot spoil unless a mold spore gets into the wound. Mold spores are light and dry, and with the slightest draught, the spores from one

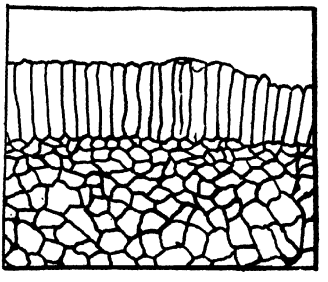


FIG. 74. Cross section of a cucumber, showing the protection of the skin by pallisade cells.

moldy orange will spread through the entire crate, even through the entire freight car. The paper wrapper keeps the spores of the molded orange from spreading, and prevents all others from chance infection.

Thirty years ago, the following story made the rounds in Washington and *se non è vero, è bene trovato.* The orange growers of California had sent their oranges for many years to New York in refrigerated cars with only a slight amount of spoilage. Then, quite suddenly, the spoilage rose to enormous proportions. Sometimes entire train loads arrived completely molded in New York. Orange growing was threatened with complete ruin by the loss of the Eastern market. An S. O. S. was sent to the Department of Agriculture in Washington. Expert bacteriologists were sent to California, and after many months of ardent research they published a bulletin stating that the cause of spoilage was largely due to *Penicillium digitatum* although *Penicillium italicum* had been found in about ten percent of all cases. And the oranges continued to spoil in transit. The orange growers were quite angry by now, and demanded other experts from Washington. Since bacteriologists had not been a great success, the government sent some economists to California. They started all over, checked all previous records, and finally came to the verdict that the oranges spoiled because of a change in the wage scale of the orange pickers. Oranges had kept as long as the pickers had been paid by the hour, and they started to spoil as soon as the men were paid by the bushel. This proved to be correct. Oranges are not picked, but cut from the tree with a knife. When the picker is hurried, as he would be if paid by the bushel, he is likely to be careless and cut into the orange. The story goes that the pickers were put back on the old pay by the hour, and the oranges arrived in good condition in New York ever afterwards.

Animal tissues are different. Only the cells of the skin have cell walls, but the muscle cells, which are the main part

we eat, have no outside protection and are helpless against bacterial invasion. However, bacteria spread rather slowly through a piece of meat. Any meat bought at the meat market will have many bacteria on the outside, from general handling, from the air, and from the chopping block of the butcher. They multiply rapidly in the rich food of the meat, and if left in the warm kitchen, a steak may begin to smell disagreeably within 24 hours. In a solid piece of meat, e.g., in a leg of mutton, the outside may be quite bad when the inner parts are still edible because the bacteria have not yet been able to work their way through the solid meat. That may require several days. It is easily seen that ground meat will spoil much more readily than a solid piece of muscle. It is not unusual to find more than a million bacteria in a gram of Hamburg steak.

Types of Food Spoilage

Spoilage of different foods follows very different paths, but certain types can be distinguished. Such a classification helps greatly in devising means for their prevention. These types are due to the chemical composition of the foods. Bacteria, yeasts and molds have very pronounced appetites, each prefers a certain diet to all others, and thus the variety of compounds which a food offers decides which variety of microbes will develop on it most rapidly and outgrow all competitors. Two compounds above all are deciding: acid and sugar, or eventually starch. Protein is present in practically all foods, and fat is of no importance, except in butter. Bacteria attack all other food compounds so much more readily than fat that the fat is still intact when spoilage is already very noticeable. Most bacteria cannot decompose fat at all. If there is spoilage, it is first noticeable by protein or carbohydrate deterioration. Fat spoilage is important only with butter which has so little protein and carbohydrate and so much fat.

THE MAIN TYPES OF FOOD SPOILAGE

Acid foods		Non-acid foods	
Containing sugar or starch	Not containing sugar or starch	Containing sugar or starch	Not containing sugar or starch
Fruits Fruit juices Sour milk	Wine, vinegar Cheese Sauerkraut Dill pickles, brine pickles	Leaf vegetables (cabbage, spinach, etc.) Root vegetables (beets, carrots, etc.) Certain fruits (melons, pumpkins, cucumbers, string beans, etc.) Seeds (grains) Flour Milk	Meat Fish, shellfish Eggs (Some leguminous seeds like peas or beans behave sometimes like this group)
Common Types of Decomposition of the Various Groups			
Molding of fruits; Alcoholic fermentation of fruit juices; Molding or alcoholic fermentation of sour milk	Destruction of acid by molds or skum yeasts growing on the surface, forming a skum	Souring: if sugar is present, formation of lactic acid; if only starch is present, formation of butyric acid	Putrefaction
Kinds of Organisms Causing the Spoilage			
Molds Fermenting yeasts	Molds Skum yeasts	Streptococcus Lactobacillus Bacterium aerogenes and its relatives Clostridium butyricum and relatives	Many different bacteria. In the absence of air, Clostridium putrificum and its relatives

With the two deciding factors, acid and sugar, four possibilities exist which are shown in the above table: acid with sugar, acid without sugar, no acid but sugar, and neither acid nor sugar. Each of these four groups contains a number of well-known foods, and the type of spoilage is characteristically different for each group.

The foods containing sugar and acid are primarily the fruits and their juices. The term fruit is used like the house-

wife uses it; the botanist may call cucumbers and string beans fruit, but to the housewife and to the writer, they are vegetables. Majority rules. All fruits spoil by molding; all fruit juices by alcoholic fermentation. These types may be followed by subsequent further decomposition, but the start is always the same, unless man interferes. To this group belongs also the sour milk and buttermilk. They contain more than 3% sugar, but it is milksugar which none of the regular yeasts can digest. Hence, molding is the common deterioration of sour milk, and usually the "milk mold," *Oidium lactis*, develops first, producing greyish-yellow islands on the surface which finally cover the complete surface with a scum so firm that flies can run on it without danger of sinking in. The mold feeds on the acid, and when all acid is destroyed, putrefactive bacteria can develop and produce bad odors.

Alcoholic fermentation of sour milk, similar to that of fruit juices, is possible, but rare, although lactose-fermenting yeasts exist. The people in the Caucasus mountains used to make kefir by placing kefir grains into fresh milk. The fermentation results in a carbonated milk containing, besides lactic acid, about 1 to 2% alcohol. The grains consist of lactic acid bacteria and a lactose yeast dried in the curd of the milk.

Spore-forming bacilli cannot grow in fruit juices and the spores of molds and yeasts are readily killed by heat. It is therefore easy to sterilize fruit juices and products made from them, such as jams and jellies, by merely heating them.

The foods of the second group represent a queer assembly of very diverse delicatessen, but they all spoil by the same method, namely by bacteria or skum yeasts or molds forming a skum on the surface and oxidizing the organic acid or, in case of wine, the alcohol. The same mold that destroys the lactic acid in sour milk, *Oidium lactis*, readily attacks the lactic acid of pickles and sauerkraut, and thereby slowly prepares the ground for later putrefaction. It is accom-

panied in this endeavor by other molds and by some very efficient skum yeasts, *Mycoderma* or *Torula*. Our picture shows skum yeast on an abandoned sauerkraut tank. Cheese which contains a good deal of lactic acid becomes moldy if not cared for.

Fruit juices become "dry" wines, i.e. wines without sugar, by fermentation, and if not protected, will be turned

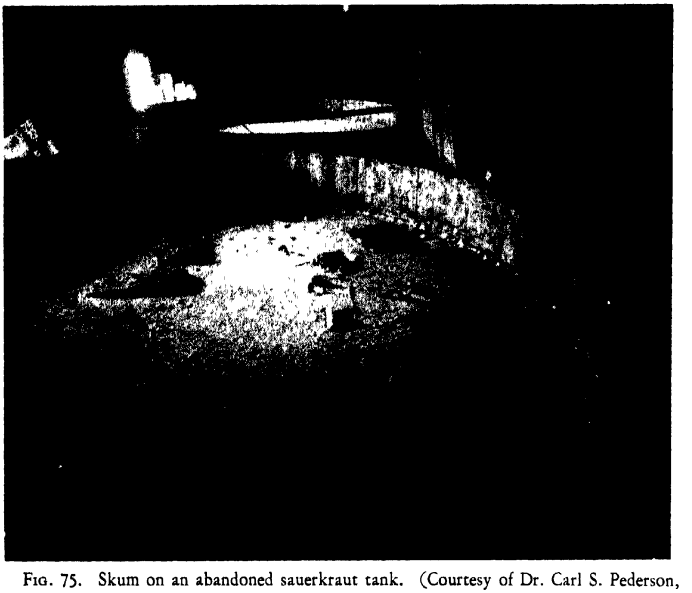


FIG. 75. Skum on an abandoned sauerkraut tank. (Courtesy of Dr. Carl S. Pederson, Experiment Station, Geneva, N.Y.)

to vinegar by the vinegar bacteria which oxidize the alcohol to acetic acid. They grow on the surface as a slippery skum called "mother-of-vinegar." When all the alcohol is changed to acid, the same bacteria, for lack of other food, begin to feed on the acid and destroy it completely, supported eventually by some skum yeasts.

All molds and skum yeasts must have air for their growth. The destruction of organic acids is a simple oxidation. It is possible, therefore, to prevent spoilage of this group of

foods by keeping out the air. This is the reason why wax is poured over jams and jellies, why Cheddar cheese is dipped in paraffine, why wine is kept in full bottles well sealed, and why sauerkraut and pickles are always kept well covered with brine.

The third group contains the foods without acid, but with some sugar or starch. This includes all vegetables, in the widest sense, i.e. in the sense of the housewife rather than that of the botanist. Pumpkins and squash and cucumbers decompose not like fruits, but like vegetables. Tomatoes are half between. They can be spoiled by bacteria as well as by yeasts and molds. This group includes also all seeds and cereals, excepting peas and beans which have so little sugar or starch, and so much protein that they usually, but not always, spoil like meat when they are wet. Only one animal product belongs in this group, namely milk.

These foods are usually spoiled by several types of bacteria, which change sugar readily into lactic acid or other acids. That raw milk turns sour, that cucumbers change into sour pickles and cabbage into sauerkraut is known to every child. Milk contains 4.7% of milk sugar of which the streptococci change not more than 1% into lactic acid, so that the sour milk still contains about 3.7% milk sugar. Most vegetables contain less sugar. Exceptions are carrots which may have as much as 9%, and sugar beets which may contain more than 16%. The sugar of vegetables is mostly glucose which differs from milksugar, but bacteria can ferment it to the same lactic acid which is found in sour milk, and streptococci are commonly present wherever vegetables are souring. Other bacteria often dominate, especially different species of *Lactobacillus* which may also occur in milk. They produce lactic acid exactly like the streptococci.

Frequently *Bacterium aerogenes*, related to *Bacterium coli*, is found which produces not only acid, but also gas. Closely related is also *Bacterium levans* of the sour dough fermentation of bread. This latter group will outgrow all other

bacteria when bread dough is made from flour and water. The dough will become light upon standing, inflated with gas which cannot escape through the viscous dough. This dough can serve as starter for sour dough bread so commonly baked in northern Europe. In the American white bread, the acid is not desired, but it would form just the same if the dough were kept for some time. To prevent this, large amounts of bakers yeast are added which develop gas rapidly and make the dough light in a few hours, before the bacteria have had time to make a noticeable amount of acid. The baking kills yeasts as well as bacteria and puts an end to further formation of gas or acid.

If flour paste stands very long, the type of fermentation may change from lactic to butyric acid. This latter acid has the very pungent and disagreeable odor of rancid butter. It is made by spore-forming bacteria among which *Clostridium butyricum* is the most common. This species is always present in soil, and finds its way easily into the flour. It can ferment starch which is indigestible for all yeasts and for many bacteria. For salt-rising bread, a culture of a certain Clostridium is sometimes employed.

Heating in boiling water kills the lactic acid bacteria, but not the spores of some bacilli. These will grow only if the lactic acid bacteria have been removed. The spores are very resistant and it is necessary to heat above the boiling point to kill all spores. In raw foods, the bacilli are rarely the cause of spoilage, though they may be present.

The pure protein foods of Group 4 consist largely of animal tissue which contains no sugar nor acid. Almost all bacteria can live on meat, and meat spoilage begins usually with a mixture of all kinds of bacteria. Quite early in this decomposition, bad smelling compounds are formed. The real putrefaction with its repulsive odors takes place only under the surface. Anaerobic bacteria which shun the air are the main factor, and most of them belong to the genus *Clostridium*. In animals, spores of these *Clostridia* are always

present in the intestine. After the death of the animal, the spores germinate and soon have started putrefaction in the intestine combined with gas formation which makes drowned animals float. As already mentioned, the same type of spoilage is encountered with leguminous seeds such as peas, beans and soy beans, because they contain so much protein.

Many of the putrefactive bacteria produce very resistant spores, and the preservation of meat and meat products is even more difficult than that of vegetables. But good observation had taught the housewives of previous centuries a simple trick, namely the preservation of meat and fish in vinegar. By the addition of vinegar, the meat changes from group 4 into group 2, acid without sugar, and this group is the easiest one to preserve. Old cookbooks recommend the preservation of meat on the farm after slaughtering by placing it in dilute vinegar and boiling it, then pouring a thick layer of tallow over the surface and letting it cool. This is probably the origin of "sauerbraten." Game is kept sometimes by immersing it completely in sour milk (buttermilk) where it will keep for a week or more, as long as the milk remains really sour. The various Scandinavian delicatessen of fish preserved in vinegar and spice can be tasted in any Smorgasbord restaurant. All these dishes originate not so much from the gourmet's efforts, but primarily from the effort to preserve. The gourmet later did what he could to improve upon the sharp sour taste of the meat or fish, and he succeeded remarkably well.

In the preceding pages, it has been shown repeatedly that bacteria produce changes in the food which need not be considered as spoilage. The souring of cabbage, if properly controlled, leads to sauerkraut. Sour milk (buttermilk) is greatly appreciated by some people. The alcoholic fermentation of grape juice will be considered spoilage by the prohibitionist, but not by the wine connaisseur. The latter considers the wine spoiled if it turns to vinegar while the prohibitionist might consider that a partial rehabilitation

of the grape juice. This reminds one of the old controversy where the American made fun of the Chinese eating "rotten eggs" whereupon the Chinese retorted that the American "rotten milk" was much worse (he referred to cheese). What is considered spoilage by one, may not be considered so by others. There is a great difference of opinion about Limburger cheese.

In all such cases, it is important that the "spoilage" is interrupted at a certain point, and prevented from progressing further. Many types of spoilage are thus being controlled to produce new types of food which are more easily kept. We have gradually developed a taste for them, and the bacteria causing such changes have been domesticated. For some such types of "spoilage," pure cultures are bred and added to the raw material to be certain of the right kind of change. The manufacture of such foods by domestication of bacteria and yeasts will be treated in the next two chapters.

Chapter Fifteen

MICROBES AND OUR DAILY FOOD

The pathogenic bacteria which cause diseases of animals or plants multiply in the live organisms. If they kill their host, they usually dig their own graves, because the dead plant or animal is then decomposed by many different bacteria, and the parasites cannot compete with the scavengers. They are easily outnumbered and die readily.

But the plants and animals which we use for food, are healthy and contain no pathogenic organisms. They do not harbor any because they can fight them off. This teaches a simple lesson for food preservation; keep the food alive as long as possible, and keep it healthy. This is done very extensively with foods of plant origin. Potatoes and sweet potatoes, carrots, beets, kohlrabis, cabbages and turnips are stored alive. They are biennial plants intended by Nature to live through the winter and bear fruit in the second year. They are not adapted to the severe winters of the Northern United States and must be protected against severe frost. But no bacteria decompose these healthy plants. Even cabbages with their roots cut off, which could not possibly grow, remain alive as long as the individual cells contain some starch or sugar.

Since these plants are alive, they respire. They take oxygen from the air into their pores, burn up the carbohydrates, and give off carbon dioxide. That means a loss in food value. It is important to reduce this respiration to a minimum, and we again follow Nature's method, and keep these vegetables as cool as possible without freezing them. We may use the root cellar, and when that is filled, we use the ancient farm method which is to dig a well-drained pit, line it with straw, fill it with the roots or tubers or cabbage

heads to be stored, and cover these with straw and earth. This gives sufficient breathing space for the vegetables, keeps them dry and the slight rise in temperature from the respiration protects them from freezing. The one danger of this simple and inexpensive preservation is the possibility of disease bacteria. Soft rot spreads easily from one root to the other, and it does happen once in a while when a pit is opened that it contains no vegetables, but a mushy, ill-smelling mass of debris. An adaptation of this old farm method to the small quantities of a city gardener is shown in the accompanying barrel storage taken from a Victory

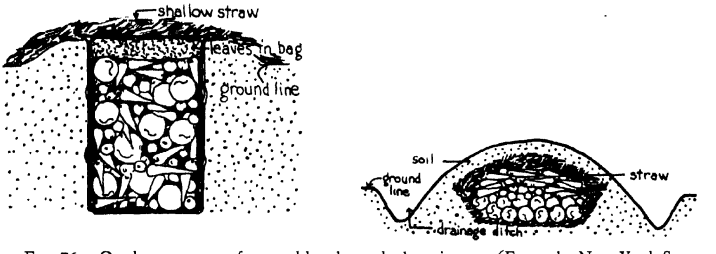


FIG. 76. Outdoor storage of vegetables through the winter. (From the New York State College of Agriculture.)

Garden Bulletin by Cornell University. For big city supplies, the root cellars have been replaced by cold storage plants with carefully controlled constant temperatures, but the principle is still the same.

The same general principles apply also to fruit. Raw fruits as we eat them are still alive. Their cells are functioning, and have respiration. They differ from the biennial vegetables listed above in one important point; they are very old cells, incapable of reproduction. They are not meant to grow; they are meant to deteriorate and give an appropriate seed bed for the germination of the seed. Therefore, they are not very resistant.

Berry fruit and cherries can be kept only for a few days, then they begin to mold. Apples must be picked by hand to prevent bruises. Oranges are carefully handled and

usually disinfected before being sent to market. Since they respire, they slowly lose their sugar content and flavor. This is prevented as far as possible by a temperature very near the freezing point, and most recently by storage in an atmosphere containing plenty of carbon dioxide which decreases the rate of breathing. They may also be coated with a thin layer of wax. Bananas are picked and shipped green. As long as fruit is not ripe, it can heal its wounds and stand a good deal of rough treatment.

Meat deteriorates so readily that it would be very desirable to store it by keeping the animals alive after they have reached the desired weight. Unfortunately, that is possible only by continuing to feed them, and as all our meat animals are warmblooded, they need more feed in winter than in summer. Considering food supply for the winter from this angle, we have apparently overlooked the possibility of domesticating hibernating animals for food purposes. Woodchucks could be fattened in late summer and fall while feed is plentiful, and then they would hibernate and stop eating when food becomes scarce. They could be slaughtered at any time during winter. Of course, they lose weight, mostly fat, through respiration. Perhaps bears could be domesticated as meat animals.

The deterioration of meat is checked by chilling. The hunter in winter hangs the deer outside of his cabin to last him for weeks. In some parts of Russia, fattened geese are killed with the first snow, and rolled into huge snowballs which decorate the front entrance of the house, until the geese are eaten. Modern refrigeration is now used in every slaughterhouse in U.S. Immediately after slaughtering, the dressed carcasses of the animals are hung in rooms which are cooled to practically freezing temperatures by a draft of cool dry air which dries the cut surfaces of the meat and retards bacterial development. All dead cells undergo a change called autolysis, or self-dissolution. The proteins become partially digested by some enzymes in the cells.

This autolysis makes the meat more tender; it is sometimes referred to as the "ripening" of meat.

Low temperatures are applied very extensively to retard the spoilage of two other animal products, namely milk and eggs. Freezing would break the eggs, but they are held as near to the freezing point as can be done without danger. Milk cooling begins at the farm, and it is kept cool until it

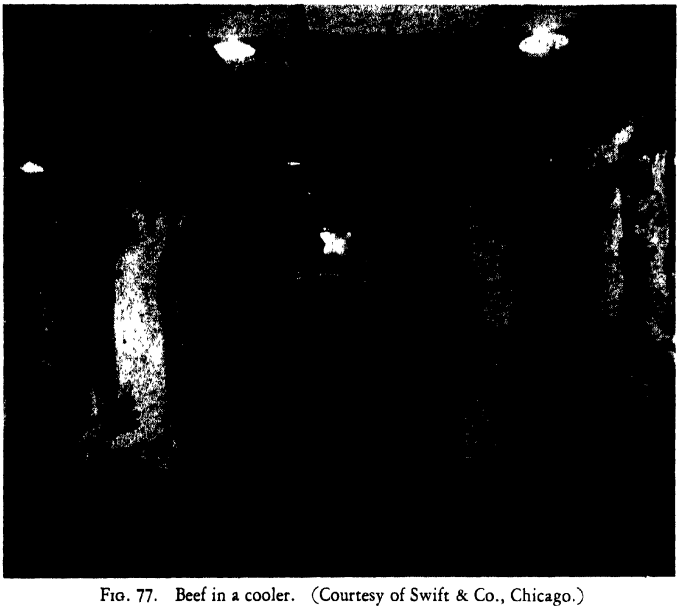


FIG. 77. Beef in a cooler. (Courtesy of Swift & Co., Chicago.)

reaches the consumer, and even the consumer puts the milk promptly in the refrigerator. It must not be believed, however, that bacteria cease to grow in the refrigerator. Many species do stop, but others multiply continually, only the rate of growth is greatly diminished, as may be seen from the picture in Chapter Four.

In these ways, we are making use of two of Nature's means of preserving food, by keeping food alive as long as possible, and by applying low temperatures to prevent

deterioration by respiration or by the growth of bacteria. The third method used extensively by Nature is the drying. Seeds are dried because they are not meant to germinate until next spring. Grass is dried to serve as food for deer and rabbits through the winter. Some animals have used this method before man appeared on earth; squirrels store nuts, grains and acorns in dry holes, and ants and bees prepare their winter supplies by concentrating or drying their food. In ancient Egypt, Pharaoh, upon the advise of Joseph, stored corn from the seven years of great plenty for the seven years of famine. This oldest type of food preservation has always remained a favorite method because of its simplicity. Although modern grain elevators contain a lot of machinery, this is not used for preservation. It has other purposes. The many million people living in Northern climates could not possibly exist without drying and storing grains of all kinds, fruits like prunes and raisins, vegetables like peas and beans, and hay and straw for the animals. Other foods like meat, fish, and leafy vegetables can be dried, but lose their palatability to some extent. Only the food shortage of the second world war could induce us to try to develop elaborate methods of drying vegetables which result in a product that gives a fairly normal taste after cooking. There was no real need before for such a method. Now, this industry has grown to enormous proportions. But milk has been dried for many years successfully, especially for cooking and baking. Egg white and egg yolk are also obtainable as well-keeping powders.

The principle of preservation by drying is very simple. In the absence of water, no active life is possible, not even that of bacteria. Nevertheless, all dried foods contain large numbers of bacteria, yeasts, and molds, which are still alive, in a dormant state, and can do no harm merely because lack of water prevents their life processes. As soon as the food gets wet, the bacteria will begin to multiply, and spoilage will soon set in. Raisins with water in a warm place

ferment in 24 hours, and flour with water soon produces a sourdough bubbling with gas which can be used as a starter to make bread dough light.

Storage in a damp atmosphere may cause some dried foods to attract enough water to start molding. Damp flour or bread or dried meat molds easily. Molds need very little moisture for development and may grow in foods which are still too dry for the multiplication of yeasts or bacteria.

There is one more way by which Nature preserves, namely by freezing. Bodies of the mastadon, the extinct hairy elephant, have been found by explorers in the eternal ice of Northern Siberia so well preserved that the dogs ate the meat. Freezing does not kill bacteria, at least not all bacteria. Enough bacteria survive to spoil foods when they thaw. It is the same as with drying; the bacteria cannot multiply or ferment because no moisture is present; all water is solid, but when it becomes liquid, bacteria will multiply again and cause deterioration.

Freezing has been used by eskimos to store fish and seal meat, and for more than 50 years, Europe has been provided with frozen meat from America and Australia. More recently, we have learned to freeze vegetables in such a way that they retain their structure after thawing. That has made freezing so popular that even in small towns and villages frozen products are kept and sold. It has been predicted that soon after the war, each household will have not only a refrigerator, but also a freezer.

The difference between refrigerator and freezer is that foods in the refrigerator do not freeze; water and cell sap remain liquid and everything retains its natural structure. Bacteria multiply so slowly that foods keep for several days, some keep for weeks, but after some time, all foods will certainly spoil. In the freezer, food will not spoil; bacteria cannot multiply in frozen materials; spoilage will set in after removal from the freezer. Freezing may change the structure of the preserved goods.

Not all food spoilage is due to microbes. Some changes are purely chemical reactions. If peaches are cut, they

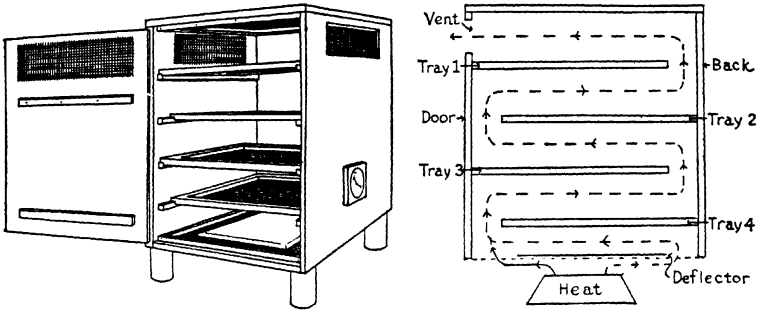


FIG. 78. Home-made equipment for drying vegetables. (From the New York State College of Home Economics.)

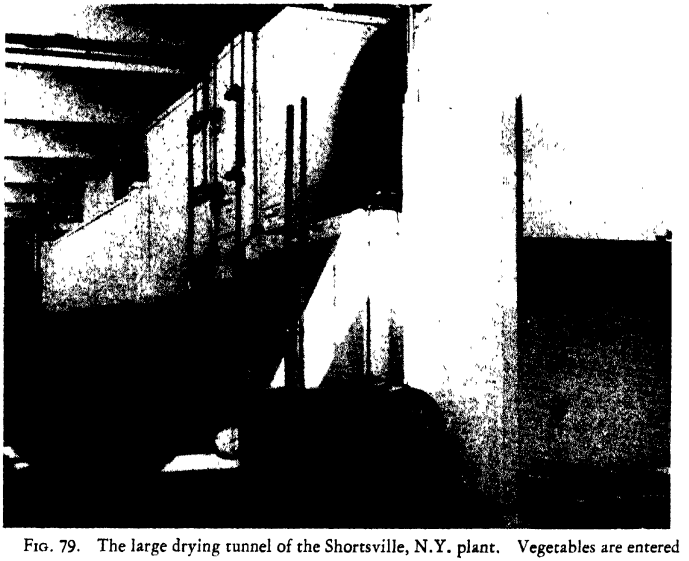


FIG. 79. The large drying tunnel of the Shortsville, N.Y. plant. Vegetables are entered at the right, and come out dry at the other end. (Courtesy of Empire State Pickling Co.)

discolor quite promptly on the cut surfaces; salsify turns dark gray; apples get brown. This is due to enzymes in the tissues

which oxidize certain constituents of the plant cells and produce the darkening. Enzymes can be inactivated by heating, and if peaches are kept under water and heated promptly, they do not become brown. Other enzymes cause a deterioration of the flavor. These enzymes continue to act slowly in frozen and dried foods, and after some months, they may taste indifferent, like hay, if the enzymes are not destroyed. This destruction is accomplished by blanching, i.e. by dipping vegetables and sometimes even fruits into boiling water for one to three minutes before canning, freezing, or drying. Even then, dried vegetables do not keep perfectly unless they are stored in an atmosphere free from oxygen, either in a vacuum or in carbon dioxide. This has been done for the last 20 years with milk powder to keep it from getting rancid.

These are Nature's four ways of preserving which man uses so extensively that more than half of our daily food is preserved either by its natural structure, or by cooling or freezing or drying. To these four ways, man has added two new, "artificial" methods, namely preservation by heat, and by chemicals.

Four chemicals are quite commonly used commercially and in the household, namely salt, sugar, vinegar, and smoke. The dry salting of fish and meat is an ancient method of preservation with those peoples who had access to large salt supplies. The salting down of beans and other vegetables is also very old. Cucumber pickles are more recent, but all methods date back to a time when bacteria were not known. Strong brine prevents the development of most bacteria, and practically all molds. A few species multiply on salt herring and salt pork and produce the characteristic flavor. A skum yeast develops on the brine of brine pickles, and must be removed from time to time.

Sugar is used to preserve jams and jellies. It does not kill the yeasts and molds, but they have not enough moisture for their life activities because sugar binds the moisture. By

the same process the bees preserve the honey. A queer mixture is the sweetened condensed milk which keeps so well that it need not be sterilized.

Vinegar, itself a product of yeast and bacterial activity, by its acidity prevents the multiplication of practically all bacteria and of many other microbes. It is used to preserve pickled vegetables, such as sweet and sour cucumber pickles, or fruit like spiced peaches or plums, but it also is employed with certain meats, like calf tongues or pigs knuckles, and the Scandinavians have many different ways of preserving fish in vinegar. The ancient trick of the housewife to preserve meat for several weeks by keeping it submerged in vinegar has been mentioned in the preceding chapter.

A preservative with a desirable taste is smoke. Smoked meat and smoked fish are preserved by a combination of several factors. Smoked pork is cured first in a strong brine, and then hung in "cold smoke." Fish are either treated in the same way, or smoked at once without pickling, first in "hot smoke" by which they are cooked, and then in "cold smoke" which gives them their taste and their golden-brown color.

Smoke consists of many different compounds, and the most efficient preservatives amongst them are pyroligneous acid and formaldehyde. But at best, smoke is only a weak antiseptic, it does not kill all bacteria, it prevents their multiplication for a long time, but not permanently. That is why smoking is usually combined with heating or drying or salting. It is not unusual to see smoked ham or smoked fish become moldy.

Chemical preservation is not very common any more. Salicylic acid and benzoic acid which were used rather commonly in the food industries of 50 years ago, are now rarely applied, and only with certain foods. The objection to their use is not so much a consideration of public health, for they are not dangerous poisons, but the improvement of methods has made their use dispensible. It is true that

they would enable the manufacturer to use partly decayed materials for his products, but microscopic tests make it fairly easy now to detect such materials in the finished goods, and definite standards have been established by the government.

The yeast industries use one chemical preservative very commonly, namely sulfur dioxide. In the breweries and wineries, the large fermenting tanks and the storage casks are fumigated by burning sulfur in them, or by using a solution of sulfur dioxide or of potassium meta-bisulfide which gives sulfur dioxide when mixed with wine or beer. By the time the liquids reach the consumer, the sulfur dioxide has been slowly changed to the harmless sulfate.

The simplest way of all food preservation would be the removal of all bacteria from the food. That is not quite as crazy as it may sound. Usually the inside of healthy plant and animal tissues is free from bacteria. Therefore, if we could only remove the contaminated outside, the remaining bulk would be sterile. This can be accomplished quite easily with bananas. If the skin is pulled down halfways with a sterile forceps, slices can be cut with a sterile knife which are dropped into sterile dishes, and can be used for the cultivation of molds and bacteria in the laboratory. The tangerine would probably be equally easy to use. Other fruits and vegetables would require more equipment. Anyhow, while this is possible in the laboratory, it does not seem applicable to a commercial process of food preservation.

The only case of removal of bacteria on a practical and commercial scale is the filtration of liquids. Special filters of different types have been constructed which can be depended upon not to let any bacteria pass (see Chapter One). Fruit juices can be sterilized in this way, by simple filtration. However, raw fruit juices become oxidized in a few days through some enzymes from the fruit, and they lose their taste and their color. To inactivate the enzymes, the fruit juice must be pasteurized. Filters are widely used for the

clarification and preservation of beer and wine before being bottled.

One means of preservation which Nature practically never uses while we could hardly get along without it, is heat. It seems a rather natural means to us because we heat most of our food before eating—which cannot be really called "natural." But since we do cook most of our food,

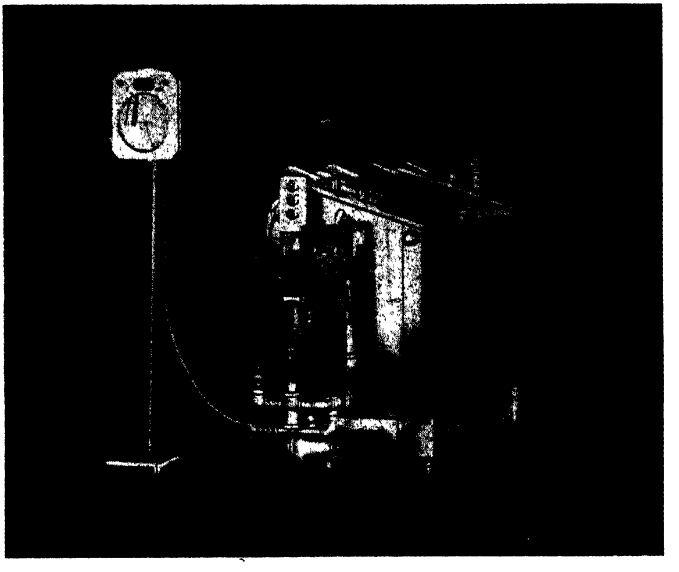


FIG. 80. Spray type Milk Pasteurizer which heats the milk, holds it at 143°F. for 30 minutes and then cools it. (Courtesy of Cherry-Burrell Corporation, Chicago)

it would be logical to do it in such a way that the food keeps ever afterwards. This can be accomplished by heating in closed containers. It was soon discovered, however, that some of our foods must be heated so long or to such a high temperature that a good deal of the flavor is lost.

Pasteur was confronting this problem when he tried to improve the milk supply of Paris. He was concerned with the rapid spoilage of milk as well as with the possibility of

disease bacteria being spread by the milk. His early experiences with sporeformers had taught him that heating until all spores are killed would impart such a cooked taste to the milk that most people would rather have no milk at all. He suggested therefore to heat the milk to about 160°F. which is sufficient to kill practically all bacteria except the spores. Since none of the disease bacteria which might possibly be in milk produce spores, such heated milk would be completely free from pathogens, it would keep much longer than raw milk, and still have a fairly fresh taste. This treatment is now known to all of us by the name Pasteurization.

At present, two methods of pasteurization are used, the "holding process" where the milk is held at 142–145° for at least 30 minutes, and the "flash process" where the milk is heated to 160–162° for at least 15 seconds. In most parts of the country, only pasteurized milk is allowed to be sold for human consumption, and it is important for the food authorities to be certain that the milk plants pasteurize the milk according to directions. Each milk pasteurizer is equipped with a recording thermometer which registers the temperature as well as the time of heating on a rotating disk of paper. The plant managers must keep these records for some time, for inspection by the health officer. Chemical tests also can be made to tell whether or not a certain milk sample has been correctly pasteurized.

It must be remembered, however, that pasteurized milk still contains living bacteria, and will not keep indefinitely, not even in the refrigerator. More heat must be employed for complete sterilization of foods, and long before Pasteur, commercial canning had been in use especially in France. It was invented in 1807 by Nicolas Appert, an Alsatian confectioner who received the prize of 12,000 francs from Emperor Napoleon for the best solution to the problem of *preserving food for the army* without salting, drying, or smoking.

Appert packed meat and vegetables in glass, corked them very tightly, and heated them in a boiling waterbath for many hours. He had the theory that foods spoiled by contact with air. The air within the container, over the food, was "rendered to no effect by the action of heat."

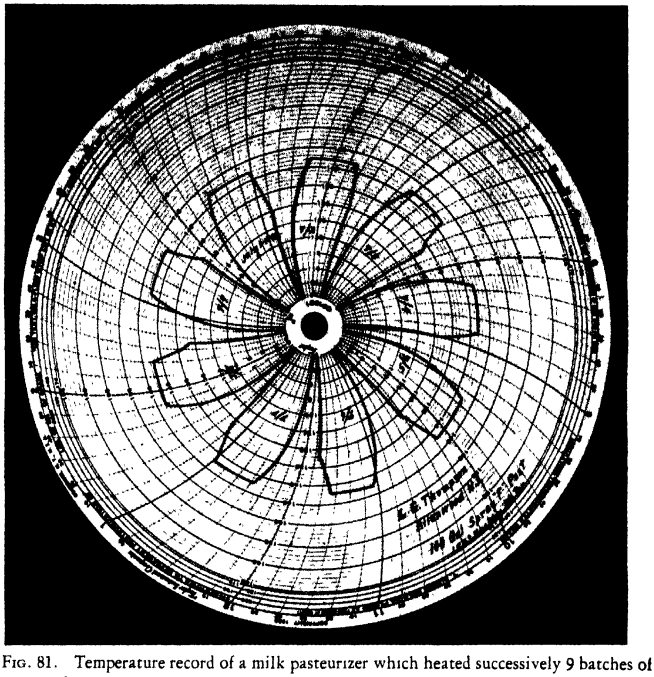


FIG. 81. Temperature record of a milk pasteurizer which heated successively 9 batches of milk to 143°F. for 30 minutes. (Courtesy of Cherry-Burrell Corporation, Chicago.)

This sounds like the theory of Needham on spontaneous generation which was discussed in Chapter Six. Appert may well have been acquainted with this theory, and his experience rather supported it. As long as his glass jars remained closed, the foods kept (though he did have considerable loss by spoilage), and soon after he had let "external air" come in contact with it, it deteriorated. A scientist

would call that a good "working theory." It was not correct, but it permitted further progress.

In England, Appert's method was soon put to work on a fairly large scale to provide ships with fresh food. Thus canned goods came to America, and soon were manufactured here too. Development was quite rapid after tin containers were substituted for the glass. Step by step, improvements were made, but prices were high, and canned goods were a luxury. Each can had to be made by hand, and soldered shut by hand after filling. Spoilage was at times quite large, especially with sweetcorn. Then it was discovered that heating in a brine bath permitted an increase in temperature, resulting in a much shorter heating time and less spoilage. Two stories are told: One story is that Isaac Solomon, a Baltimore canner, had learned that Sir Humphry Davy fifty years ago had found that calcium chloride increased the boiling point of water, and he applied this knowledge to canning, and told others about it. The other story is that some clever business man had somehow found this out, and sold to the canners a compound of secret composition which when put into the waterbath would mysteriously protect the food inside the tin from spoilage. The mysterious compound was commercial calcium chloride, sold for many times its market price.

An autoclave for pressure cooking had been invented in 1852 by the son of Appert, but it seems that this knowledge never reached the United States and only in 1874 was the first "retort"—that is the canners' name for pressure cooker—built and used. But the salt bath and also the oil bath remained in use for a long time, side by side with the retorts.

To decrease the time of heating with such pasty materials as cream style corn or pumpkin, agitation during heating was made possible, either by making the cans roll through the heating bath, or by a shaking machine inside the "retort." Thereby, the time of "processing" i.e. of heating could be cut considerably.

The greatest stimulus for the canning industry came through the development of the tin can. First, the cans became much cheaper by being made in quantity by elaborate machinery. Then, a method was worked out to close the can by placing a loose cover on the can which then is folded by a machine onto the edge of the can by a double seam.

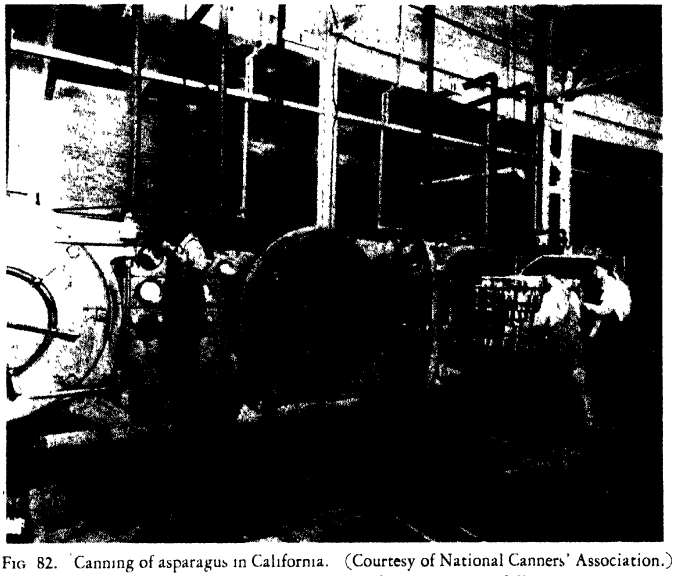


FIG. 82. Canning of asparagus in California. (Courtesy of National Canners' Association.) At right, the retort is being filled, in center, it is full. At left, it is closed, and steam pressure is applied.

This permits easy filling of the wide open can, and does away with all soldering. This was climaxed by having this closing performed in a vacuum chamber so that all air is removed from the can before it is heated. This brings about a great improvement in flavor and in color with some of the more sensitive fruits and vegetables.

Like all other food industries, the canning industry is closely watched by federal authorities to insure wholesome goods.

Before canning, the products to be canned are sorted and washed. Then begins the preparation for canning. Some foods are peeled, like beets or peaches, some are cut like apples or carrots, most vegetables are blanched by dipping in boiling water for a minute or two before being placed into the cans.

The machinery for preparing the food for the cans with a minimum of human contact is remarkable. The prize belongs to the pea viner, a machine which takes the entire pea plant as it is cut in the field, picks off the pods, cuts the pods open and takes out the green peas.

The 3,000 canning factories of the United States protect a very large amount of food from bacterial invasion. The record of the last years before the war showed in round numbers the following output:

Milk, condensed and evaporated	50	million	cases	of 24	No. 2	cans
Tomatoes and tomato juice	38	"	"	" "	"	"
Peas	24	"	"	" "	"	"
Corn	24	"	"	" "	"	"
Peaches	14	"	"	" "	"	"
Pineapple and pineapple juice	20	"	"	" "	"	"
Beans, green, wax and lima	10	"	"	" "	"	"
Grapefruit and grapefruit juice	10	"	"	" "	"	"
Other vegetables	15	"	"	" "	"	"
Other fruit and juices	28	"	"	" "	"	"
	233					

This is a total of over 5 billion cans. It does not include meat and fish. Their records are not given by cans, but by pounds. The annual meat canning in peace times amounted to nearly 200 million pounds, the canning of fish and shellfish to about 450 million pounds. It is a remarkable and a rather amusing fact that next to milk, the largest number of cans each year is not used for tomatoes, but for dog food.

Certain sporeforming bacteria do occasionally survive the ordeal of being "processed in a retort," and germinate and multiply afterwards, decomposing the supposedly steri-

lized food. Some develop gas and cause the can to swell and eventually even burst. Others make only a little acid, and this spoilage is called "flat sours." Also, each type of food has its particular troubles.

The organizations interested in canning, such as the National Canners' Association, the larger can manufacturers, and a number of Agricultural Experimental Stations have a number of bacteriologists as trouble shooters as soon as spoilage becomes known. It is rather interesting that the scientific work of the canning industry is mostly not done by the individual companies, but by their collective research institute, the National Canners' Association, and also by the companies providing the cans and the machinery. Thus, the canner remains an artist rather than a scientist, striving for highest quality within the rules set by the scientific expert.

Chapter Sixteen

THE COMPROMISE ON FOODS

Since bacteria have no brains and cannot be taught to distinguish between the organic matter which man wants to keep as food, and the organic matter which man considers waste, there has been a continuous conflict. Finally, man has agreed to a compromise by permitting some microorganisms to decompose certain foods to some extent, but not completely. The partly decomposed fruit juices and grain extracts which we call wine and beer, have already been discussed, and it was shown that only very few specialized yeasts have the privilege of this compromise while all others are rigidly shut out.

This chapter shows that not only yeasts, but also some bacteria and some molds are permitted to decompose certain foods. The main products of this partial decomposition are sauerkraut, pickles, ensilage, butter, cheese and vinegar. All decompositions are interrupted when a certain stage is reached, and no further deterioration is allowed and this stage is determined largely by the appeal of the food to the human taste. This primary decomposition results in every case in the formation of acid, and the acid foods are more easily preserved.

As in most cases of food preservation, it was not the scientist who thought up these clever methods. They were in general use a few thousand years before bacteria had been discovered. The manufacture of wine, vinegar, cheese and butter had been worked out by the time-honored method of trial and error; these foods are mentioned as standard goods in the Bible. Very old, although perhaps of European rather than Asiatic origin, are sauerkraut, pickles, and silage. The so-called "rotten eggs" of the Chinese probably belong in this class of foods preserved by partial decomposition.

At the time when bacteria were finally recognized as the cause of organic decomposition, large cheese factories, sauerkraut factories and pickle factories were in operation and turned out products of a very high quality. The bacteriologists who came to the factories to study the bacteria causing the cheese to ripen or the cabbage to turn sour were merely tolerated by the industry, and the experienced managers smiled at the peculiar work of these men who knew none of all the trade secrets upon which superior quality depended. In most cases, there was little cooperation, but usually a good-natured tolerance.

The change in this relationship came, as a rule, whenever a sudden unexpected or unprecedented spoilage occurred. When the cheese produced so much gas that it was blown to pieces, when the pickles became slippery or the sauerkraut turned pink, the manager was at a loss what to do. The bacteriologist recognized the trouble as being due to a new kind of bacteria which had entered the factory. He could either find a method to suppress them without hurting the other, desirable bacteria, or he could find out where the troublemakers came from, and keep them out of the plant, or he could sterilize everything and start anew by using pure cultures.

As an example, the following story happened in 1910 in the pickle industry of the Middle West. At a certain salting station, all brine pickles became mushy, they were utterly unsalable. The manager tried all tricks of the trade that he could think of, he even brought water in tank cars from another salting station that produced perfect pickles, but it was without avail. The pickles turned soft again. Finally when the old experienced craftsmen could not help, the manager went to the State Agricultural Experiment Station. The chemist could find nothing wrong with the salt, and sent him to the bacteriologist who promised to investigate the spoilage. That was in February when normal pickling cucumbers could not be had. The investigation

had to be postponed till summer, and then the bacteriologists learned to their surprize that brine pickles underwent a regular fermentation process, and that only after this, would they keep. Nobody had studied brine pickle fermentation, and the dill pickle process which had already been studied in Germany, was quite different. The entire summer was needed to get acquainted with the *normal* process before the *abnormal* process could be understood. Another winter went by when little could be learned because there were no cucumbers. When toward the end of the second summer, the bacteriologists had mastered the problem and felt fairly certain that they could prevent pickles from getting mushy, they could not find the manager who had started the investigation. His losses had been so heavy that the salting station had been abandoned. But other pickle factories were glad to get this scientific information which they found soon to be very practical.

In this way and through similar occurrences, the plant managers, with their great store of practical experience, and the bacteriologists, with their biochemical reasoning, finally learned that the two together could accomplish more than each one separately. Industry began to cooperate with science, and the result is the present high development of our various food industries.

The first great advances in industrial management were made in the breweries, thanks to the efforts of Pasteur and especially of Emil Christian Hansen. The dairy industry did not really become bacteria-conscious until about 15 years later, and this development was soon followed by thorough investigations of the souring of various vegetables.

The simplest case is that of sauerkraut, simple from the manufacturer's viewpoint and simple bacteriologically. Cabbage, like most vegetables, contains a small amount of sugar, usually between 3 and 5 percent of the total weight. That is not very much, but sugar is such a good food for bacteria that as soon as cabbage is shredded and the juice

comes out of the cut cells, the bacteria which are always present on the leaves will feed on it. At first, many different bacteria multiply because there is plenty of food, and cabbage contains a little protein besides the sugar, but after one day of unrestricted multiplication, so many bacteria are present that competition begins.

FIG. 83. Sauerkraut manufacture. Rapidly rotating fan-shaped knives cut a head of cabbage to uniform shreds in half a minute. (Courtesy of Empire State Pickling Co., Phelps, N.Y.)

An important phase of kraut manufacture is the pressing down of the shredded cabbage to the smallest space possible in order to keep the air out. This is accelerated by salting. Only enough salt is added to give the right taste, and that is not enough to injure the bacteria. But it draws the moisture out of the leaves and makes them limp and wilted, so that they can be compressed more easily. All cells of the cabbage leaves which were not destroyed by the shredding die under these circumstances and offer no further resistance to bacterial attack. In the lower layers of this compressed

FIG. 84. Sauerkraut Manufacture. Shredded cabbage, dumped in huge tanks, 14 feet deep, holding 125 tons, is salted by a man in rubber boots. (Courtesy of Empire State Pickling Co., Phelps, N.Y.)

cabbage, the little air caught between the shreds is soon used up by the bacteria, and from then on, only those types can multiply which need no air. Prominent among these are certain lactic acid bacteria, streptococci as well as lactobacilli, and these grow by fermenting the sugar of the cabbage largely to lactic acid. Thus the cabbage becomes sour, oftentimes more strongly sour than sour milk.

When all sugar is used up, no further decomposition can take place below the surface. The kraut is now ready for consumption. On the surface, where air has access, molds and skum yeasts can multiply and oxidize the lactic acid completely, to water and carbon dioxide. This slow, but complete destruction of the acid by the respiration of molds or yeasts leaves the shredded cabbage without its protective acid, and then, putrefaction can set in, and the kraut becomes brown and slimy, and develops a bad odor. This is prevented in the household by pressing the kraut under the brine with a plate which is weighted down by a stone. Any stone will do except limestone which is decomposed by the acid of the kraut. In the factory, each tank is covered with tight-fitting boards which are pressed by cement blocks into the kraut just deep enough to prevent contact of the kraut with the air.

The bacteria which cause the desired souring are always present on cabbage leaves, only in small numbers, but capable of multiplying rapidly when conditions are favorable. A housewife making her own kraut has no difficulty getting the right acidity if she keeps the air out and does not oversalt the cabbage. In industry, the procedure is quite as simple. Rotating knives are used for shredding, and otherwise the manufacturer lets nature take its course. No pure cultures are used because they are not needed. Wooden tanks, some of them holding as much as 70–80 tons of cabbage are used exclusively. The salting in these deep containers is done by a man in rubber boots who

tramples down the cabbage to press out as much air as possible between the leaves.

For shipment to the consumer, the kraut must be removed from the big tanks into smaller barrels. This involves air contact which is continued when the kraut barrel stands in a cool corner of the grocery store. If the sale is rapid, there will be no spoilage. But most of the sauerkraut manufactured commercially is now put in tin cans and preserved by heat like canned fruits.

Similar to the sauerkraut industry is the pickle industry. We must distinguish between dill pickles and brine pickles which are made in different ways. Dill pickles are usually made in small barrels. The barrel is packed tightly with cucumbers, and on top of these are placed a variety of flavored leaves such as grape leaves, cherry leaves, or horse radish roots, even a little garlic is used by some people, and always a certain quantity of dill weed or dill seed. Then the barrel is headed up, turned on the side, the bung is taken out, and the barrel is completely filled with brine of not over 5% salt through the bunghole. The brine will draw some juice out of the cucumber containing a little sugar, and all kinds of bacteria begin to grow in this brine until the air is exhausted. Then the same thing happens as in sauerkraut; most bacteria stop because they need oxygen, and some lactic acid bacteria continue to grow and ferment and soon make the brine so acid that no other bacteria can exist. During the first few days, the mixture of bacteria causes a slight gas formation. As soon as that ceases, the bung is hammered in the hole, and the last trace of air is shut out.

In these barrels, no further bacterial decomposition can take place, as long as the air is kept out. However, the barrels are not very large, they are not absolutely tight, and air slowly penetrates through the pores of the wood. In this way, the acidity gradually decreases, for on the cucumbers are always a few skum yeast cells and molds waiting for a little oxygen which permits them to grow by oxidizing a

little of the acid. Finally, a point is reached where the acidity becomes so low that either the skum yeasts themselves or some bacteria other than the lactic bacteria attack the pickles. At first they get slippery, and finally so mushy that the fingers go right through the pickle when one tries to take them out of the barrel. Air-tight barrels and storage at low temperature greatly prolong the life of dill pickles. They can also be preserved by pasteurization in airtight tins in their own acid juice.

Brine pickles are made differently. They are put up in large open tanks which in the old times stood outdoors and not under a roof. Even now, some tank stations have no roof. The cucumbers are placed in strong brine containing 10 to 15% salt, or 80 to 120 pounds of salt per 100 gallons. The cucumbers float in this brine, and must be forced under the surface by a wooden rack. They shrink greatly, and only very slowly do they finally regain their original shape. In this strong brine, most bacteria cannot thrive at all, and even those which finally bring about a lactic fermentation multiply very slowly. But in the end, the result is the same as in the dill pickles. So much lactic acid is formed that no other bacteria can grow, and the cucumbers keep as long as the acidity is maintained. The author has seen in a pickle factory a certain size of brine pickles held for 7 years without spoilage.

On the surface of these large tanks, molds cannot grow because of the salt, but certain skum yeasts do not mind the salt and will form a skum one to two inches thick when left undisturbed. They feed exclusively on the lactic acid. As the tanks are very deep, it would take a long time before all the acid is decomposed. Of course, the skum is not left undisturbed. It is frequently skimmed off, but the remaining cells multiply quite rapidly forming a new skum.

It is interesting to note that the tanks which stand in the open do not develop a skum. The sunlight is strong enough to kill all yeast cells that get started. Thus we have the

strange result that brine pickles in outdoor tanks exposed to dust and insects, rain and snow, keep longer than inside the pickle plant. With covered tanks, an ultraviolet lamp over each tank can be substituted for the sunlight. This keeps the yeast growth down, but it is too expensive a process.

The brine pickles must be put in fresh water for several days to leach out the excess salt before they can be manufactured into sweet pickles, sour pickles, or "American dill pickles," as they are called in contrast to the genuine or "German dill pickles." The American dill pickles are made sour with vinegar, and dill oil and some other spices are added for flavoring.

Several other vegetables are treated similarly in strong brine, especially cauliflower, tiny onions called chalottes, peppers and green tomatoes. They are used either as such or as mixed pickles, sour or sweet. Their bacteriology is in no way essentially different from that of the brine pickles.

This principle of preserving vegetables by permitting them to sour, and preventing all further decomposition by excluding air or by other means, is applicable to any vegetable containing at least 1% sugar. It is used occasionally to preserve snap beans, green peas, red beets, tomatoes and even apples. Usually, however, beans are preserved by a process similar to that of brine pickles, with large amounts of salt that must be leached out to make the beans edible.

A large scale application of the same principle is the manufacture of silage for farm animals. Silage is a kind of sauerkraut made from corn plants, grass and other forage plants, by the same principle of permitting it to sour, and preventing all further decomposition as far as that is possible without much expense and labor.

These vegetables are much easier to keep after they are soured, and that is of course the main reason for letting deterioration go that far. The change of taste connected with this souring was not the primary reason for manufacturing these fermented foods. The taste for sour vegetables

was gradually acquired, and not by all people, although the manufacture of 150,000 tons of sauerkraut per year in the United States alone seems to indicate that many people must like it.

This souring is possible because the vegetables contain a little sugar. Meat would also keep if it were sour, but no natural fermentation is possible as meat contains no sugar. However, centuries ago the farmers' wives had already observed the preserving qualities of acids, and after the slaughtering of a pig or beef, they covered a nice roast with vinegar in a deep jar and in that way preserved it for a week or two before cooking. Such a roast tastes distinctly of vinegar, and is not to everybody's liking while others consider it a special treat.

The manufacture of vinegar will be discussed in a later chapter.

While the ancient people who tilled the soil developed methods for preserving their vegetables, the nomad tribes with their herds of cattle invented means of preserving the milk. It cannot be doubted that the milk carried in goat skin bags on the backs of horses or donkeys soured very rapidly, and many different types of sour fermented milk have come to us from the Balkan countries and Asia minor. The various names given to different kinds of sour milk, such as Matzoon, Kefir, Kumys, Yoghourt, suggest that they are considered as an important part of the diet. All of these fermented milks are strongly sour from lactic acid, but the acid in each is produced by different bacteria, and these bacteria develop different additional flavors. Some of these drinks are made from raw milk, some from boiled and slightly condensed milk, and Kumys contains a yeast which produces a little alcohol and enough carbon dioxide to make the milk effervesce.

Another type of sour milk which was developed after the style of Matzun and Yoghourt, but with different pure

cultures, is the so-called Acidophilus milk which will be discussed in Chapter Eighteen.

In spite of much advertising, these fermented milks have not become very popular in this country, neither the "high-brow" acidophilus milk developed by scientists nor the "low-brow" types from the Balkan countries. They are mostly used on prescription by the family doctor. People who like sour milk commonly resort to buttermilk. The buttermilk sold in American cities is usually not buttermilk in the true sense of the word, it is not the liquid left over

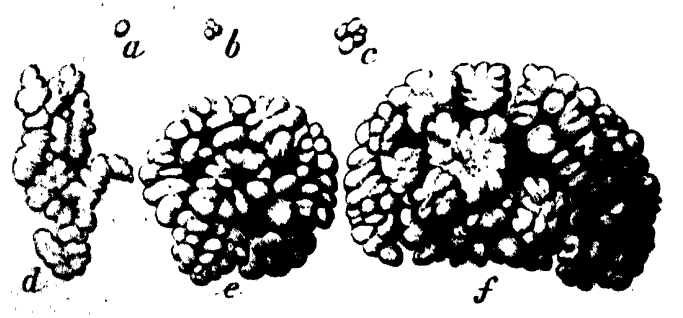


FIG. 85. Kefir granules, consisting of bacteria in curd, change milk to kefir. The granules increase in size and numbers during the fermentation. (From Lafar, Technische Mykologie.)

when the cream is churned and the butter is removed. Buttermilk for city consumers is pasteurized milk or skim milk that has been soured by pure cultures of bacteria specially selected for their good flavor.

There are two reasons for the manufacture of "cultured" buttermilk instead of using the natural buttermilk. One is the great specialization of the dairy industry. The densely populated areas of the country need so much fresh milk that for hundreds of miles, not enough milk is left over for churning. The butter factories are all in the purely agricultural areas with wide stretches of pasture land whereas the people who want buttermilk are mostly in the big cities.

Besides, butter manufacture has changed from sour cream butter to sweet cream butter. In 1895 or near that time, the dairy industry started to pasteurize the sweet cream and ripen it, i.e. sour it, with selected aroma-producing pure cultures. That butter kept much better than the butter from naturally soured cream, because it did not contain *Oidium lactis* or *Cladosporium butyri* or *Pseudodomonas fluorescens* which split the butterfat and make the butter rancid.

The butter surplus of the summer was kept in cold storage for the winter. The cold storage industry developed greatly after 1900, and while the butter was kept near 32° in 1895, it was kept near 25° in 1905, and near 0° in 1915. No bacteria could possibly grow at zero, and yet, the butter slowly spoiled. It did not become rancid, but it turned fishy, and it was soon learned that only sour cream butter became fishy, and not the sweet cream butter. The acid reacted with some other milk constituents, and made trimethyl amine which smells like salt herring. Naturally, all large butter factories changed their method and churned sweet cream butter, from pasteurized cream. By this time, they had learned enough bacteriology to keep out the organisms which cause rancidity. The buttermilk from sweet cream churning does not taste very good, and it would not pay to ship it a long distance to the centers of consumption.

Oleomargarine is made from plant or animal fats which are churned up in soured skim milk and then chilled by squirting the warm mass into ice water. All souring is done with special pure cultures in pasteurized skim milk.

The most important compromise with bacteria has been made by the cheese-makers who would be helpless and out of a job without microbes. Even such a lowly product as cottage cheese cannot be made without them. When sour milk is stirred and slightly heated, the solid curd contracts and sinks to the bottom, and a cloudy, slightly yellowish liquid is on top which is called the whey. The curd contains most of the protein and fat, and as this is the most

valuable part of the milk, from the nutritional viewpoint, efforts have been made to save it for food. This curd is known to us as cottage cheese. It is usually prepared from pasteurized skim milk which is soured with pure cultures of lactic acid bacteria. When the milk has coagulated, the

FIG. 86. Old instruments still in use for cutting the curd in cheese-making. (From Fleischmann: Das Molkereiwesen, 1876.)

curd is cut or stirred to break it up in small units, and then the milk is heated slightly to make the curd contract to small granules. When it has settled, the whey is drained off, the curd is washed with a little water to prevent it from getting too acid, and then it is ready for consumption. Cream can be mixed into it to make a product known in the Eastern States as Philadelphia cream cheese.

The large, solid cheeses which the dairy man calls hard cheeses, like American, Cheddar, Swiss, and Edam, are made quite differently. The milk is not curdled by acid, but by rennet which is the same thing as junket powder. The old cheesemakers extracted the rennet from calves' stomachs by keeping them in acid whey. Today, rennet manufacture is a special industry, and the cheesemaker buys rennet as powder or as liquid extract, with a guaranteed strength. A measured quantity of rennet is stirred into sweet milk, and after from half an hour to one hour, depending upon the kind of cheese to be made, the milk is coagulated. It is then cut with a long knife and stirred with the "harp," a frame with several thin parallel wires, which cuts the curd uniformly into small pieces. Then the milk is warmed slowly, quite high for Swiss cheese, less so for American Cheddar, very little for Camembert. The curd contracts correspondly, very much for Swiss cheese, very little for Camembert. This curd is dipped out with sieves or ladles, and placed in wooden molds lined with cheese cloth from which the whey can run out through many holes, and in a few days the cheese has settled sufficiently so that it keeps its shape when the mold is removed. From the very beginning of the treatment of the milk, certain bacteria in the milk have multiplied, above all the acid-forming species, but also a few others. They continue to multiply in the cheese, and it is largely due to their action that the cheese ripens and develops its characteristic flavor and texture, though the enzymes of the milk and of the rennet contribute also to the change of the casein.

The different types of cheeses are produced by different treatment which favors different groups of bacteria. For Swiss cheese, very fresh milk is used, stirred to very fine curd particles and heated quite high before the curd is dipped out. This makes the cheese dry, kills some common acid formers, and favors other species. For Cheddar cheese, the milk must be slightly acid. Usually, acid bacteria are

added. These two types of "hard cheese" are rubbed with salt, or put in strong brine, and later they are paraffined in order to prevent bacteria from growing on the surface. They ripen only on the inside; the rind is not eaten.

Soft cheeses like Limburger or Camembert are also made with rennet from fresh milk, but after they come out of the form, they are salted but very lightly. With Limburger,

FIG. 87. A primitive Swiss cheese factory in the Alps.

bacteria together with some mycodermas grow on the surface, and gradually destroy the lactic acid on the outside and produce a brownish, slippery surface by digesting the casein. This results in the strong odor of this cheese.

The milk for Camembert cheese is inoculated with spores of a mold, *Penicillium camemberti* which rapidly destroys the lactic acid on the cheese surface, together with *Oidium lactis*, and attacks the casein, producing the flavor which is so typical for this cheese. The gray-green mold is plainly visible on the surface. If overripe, the casein

will be partly broken down to ammonia. The rind of these soft cheeses is eaten.

Roquefort cheese is a combination of hard and soft cheese. When the curd is cut, it is mixed with a small quantity of "moldy bread." At the town of Roquefort in France, the bread is permitted to mold in certain natural caves. In this country, spores from a pure culture of *Penicillium roqueforti* are substituted for the moldy bread. The cheese, almost the size of a Cheddar cheese, would give the

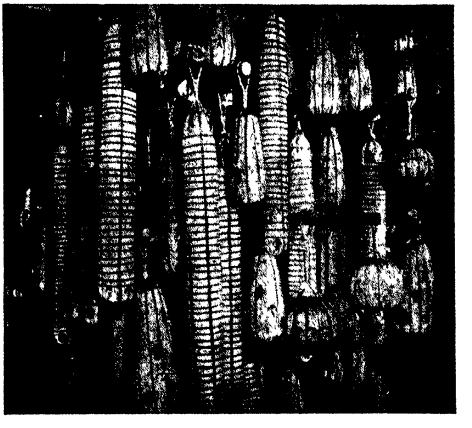


FIG. 88. Different types of Italian cheeses.

mold little chance to grow, especially as the surface is rubbed with salt to prevent any outside development. But the cheese is pierced in many places with long needles, and thereby, air can get into the interior, and molds grow along these air channels and produce the greyish-green spores which give the typical mottled appearance to Roquefort cheese. This mold attacks the fat as well as the casein and thereby brings forth the Roquefort flavor.

Of the many different types of cheeses produced in the various countries of Europe, we have adopted only a few, but England, France, Germany, Italy and the Scandinavian countries have each a large selection of standard makes of

cheese. These differences in taste and structure are brought about by varying the acidity of milk used, the amount of rennet and the temperature of coagulation, the size of the curd particles, the temperature of heating after the curd is cut, the size of the cheese, the treatment of the rind, the amount of salting, and the time and temperature of curing. All these factors decide which of the many different kinds

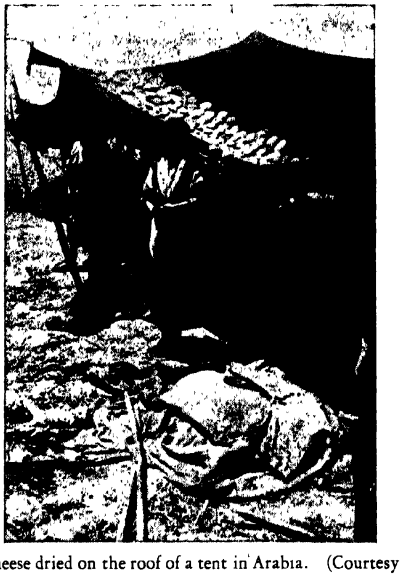


FIG. 89. Cottage cheese dried on the roof of a tent in Arabia. (Courtesy Agr. Experiment Station, Geneva, N.Y.)

of bacteria normally present in milk will develop best, and how far they will develop. All of these types were established before bacteriology was ever applied to dairying.

How new types originate, may be learned from the "invention" of Tilsit cheese. About 60 to 70 years ago, the rich pastures of East Prussia attracted the interest of Swiss cheese makers. There seemed to have been an overproduction of cheese makers in Switzerland at that time, or perhaps a local economic depression made them leave their home country. Anyway, quite a large number of them settled

in the eastern-most part of East Prussia, between the cities of Tilsit and Memel on the Baltic Sea, and started to make Swiss cheese. Milk was plentiful, cheese after cheese was made, but when, after 6 months, the first cheeses were ripe, they were not Swiss cheese at all. They did not

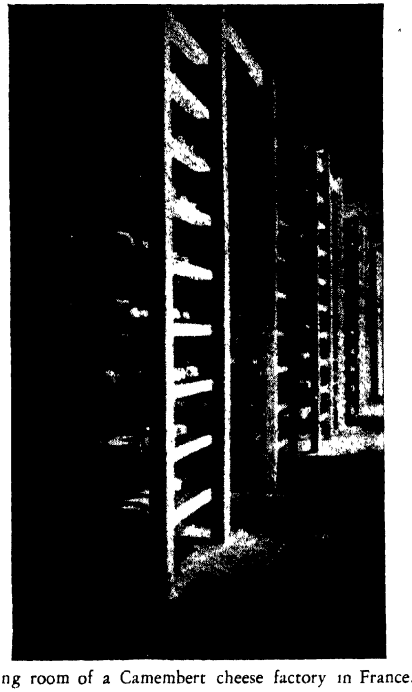


FIG. 90 Ripening room of a Camembert cheese factory in France. (Courtesy of Agr. Experiment Station, Geneva, N.Y.)

have big round "eyes" as the holes are called, but plenty of little slit holes; the curd was not dry and did not have that peculiar sweetish taste characteristic of the home product; the cheese was moist, rich, and the taste was entirely different. The cheese makers were perplexed. Some went back to Switzerland, but the more resourceful ones reasoned that there must be a market for this cheese which was not a

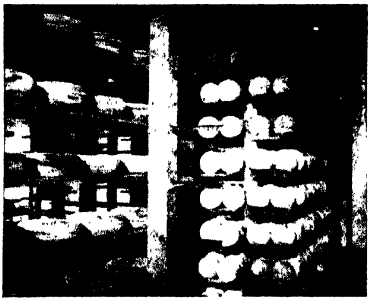


FIG. 91. Gouda and Edam cheese, ripening, in Holland. (Courtesy of Agr. Experiment Station, Geneva, N.Y.)

bad cheese although it was certainly no Swiss cheese. Gradually, a market for this kind of cheese was developed, under the name of Tilsit cheese, and throughout Northern Germany, it is now a favorite cheese.

Since then, the cheese makers in East Prussia have learned to make real Swiss cheese too, with big "eyes" and perfect taste, thanks to the bacteriologist who can provide them with a pure culture of *Propionibacterium Shermanii* which makes the gas that produces the holes. Now-a-days, when milk is obtained in a much cleaner way than 50 years ago, it contains far less bacteria, and the cheese

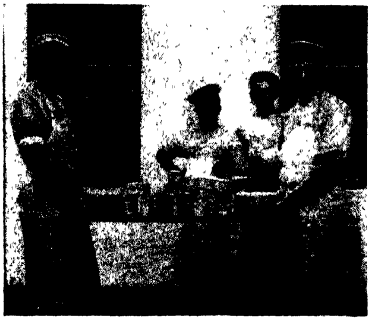


FIG. 92. Münster, Limburger and Brick cheese in a German dairy school. (Courtesy Agr. Experiment Station, Geneva, N.Y.)

maker cannot always be sure that the bacteria which he needs to ripen his cheese are present in sufficient numbers. He therefore relies more and more upon pure cultures of bacteria which he adds to the milk or the curd. He can buy cultures for acidifying the milk for American or Cheddar cheese; for producing the brownish, slimy layer of Limburger cheese, together with its strong flavor; for making the holes in Swiss cheese; and for producing the right flavor of Camembert and Roquefort cheese.

FIG. 93. Commercial cultures for butter and cheese making.

CHAPTER SEVENTEEN

MICROBES, THE FOOD OF THE FUTURE

Two entire chapters have already been devoted to the role of microbes in our nutrition. In one chapter were discussed the means of keeping microbes completely out of our food; in the other, we compromised by letting some of them grow in our foods for a while. Now comes the third

step. We not only let them grow for a short time, but we let them multiply to their limit, permitting them to decompose the food completely—and then we eat the microbes.

It is too late to shudder at the idea of eating microbes because we have been doing it all our lives in our regular meals. All our bread is baked with yeast, and yeast will probably be the most common microbe of our future diet. We digest the lactic acid bacteria of cheese, buttermilk and sauerkraut, and with soft cheeses like Camembert and

Roquefort, the aroma is produced by molds, and the mold mycelium is part of the cheese. We eat mushrooms which are fungi very similar to the molds.

But you will ask the question why we should change from our present and pleasant diet of fruits, vegetables and meats to microbes. Let us understand from the start that microbes will be eaten only as supplementary food, and the chemical composition shows plainly that they cannot substitute for fruits or vegetables, they can only take the place of meat. They consist, besides water, mainly of protein which is also the main constituent of meat.

AVERAGE COMPOSITION OF MICROBES

	Bacteria	Yeast	Mold mycelium	Lean beef
Water	75%	75%	80%	67%
Protein	14%	12%	8%	19%
Fat	3%	3%	2%	13%
Ash & Cellulose	8%	10%	10%	1%
Protein in One Pound of Dry Material				
In ounces	9	7½	6½	9½

Food values are usually compared on the dry basis, and our figures show that microbes are not much inferior to meat, as far as protein is concerned. Most of the microbes also contain the water-soluble vitamins, often in much larger quantity than we find them in meat. Thus it must be admitted that microbes could be used as meat substitutes, if we have to substitute or if we wish to do so.

There are three reasons which will induce people to eat microbes in place of meat: (1) if they cannot get meat; (2) if microbes taste better than meat; (3) if microbes are cheaper than meat.

The war has brought us close to the first condition. Meat became scarce, and substitutes had to be found. Aside from soy beans, the most popular substitute was yeast.

The brewers' yeast is a waste product after it has accomplished the fermentation. It used to be dumped in the sewer. Now it is "debittered," i.e. the bitter compounds of the hops are removed, then it is washed and dried, and the dry brewers' yeast is an excellent substitute for meat.

The amount of brewers' yeast is limited by the beer consumption, and the quantity is not nearly sufficient to supply the demand for concentrated protein foods. If we expect to go on a yeast diet, the yeast will have to be grown especially for food. Our knowledge of yeast cultivation is excellent, from our experience with bread yeast, and it is unquestionably possible to grow large quantities of yeast. Whether this will be done in the future, depends upon the other two factors mentioned, the taste and the price.

It is possible to prepare from brewers' yeast a yeast extract which is equivalent to meat extract in food value, and similar to it in taste. Yeast extracts have been manufactured as substitutes for meat extract, and have been used for many years for soup stock, gravies etc. Recently a variety of yeast has been discovered which produces the natural flavor of meat. It has been named *Torula utilis*. It may require a little time, but the problem of making yeast into an appetizing and appealing dish will certainly be solved.

More difficult is the price question. The price of bread yeast is too high to make it a cheap substitute for meat, but a good share of the high price is probably due to the present method of selling it packaged in very small quantities, of keeping it refrigerated, of discarding old yeast cakes, and so forth. If instead, yeast could be merchandized as dry powder in pound packages similar to those for cereals, without refrigeration, the cost would be considerably less.

It may seem surprising that we can think of producing protein cheaper than nature, but yeast growth is accomplished by nature, not by us. All we can do is to provide the best conditions for growth. Growing yeast for food

would be merely shifting from higher animals to simple organisms. Nobody can claim that our present methods of growing meat animals are natural. The concentrated food which the pigs and cows get, is very different from their natural food. Cows which give 30 quarts of milk a day are monstrosities produced artificially by breeding for a very onesided purpose. Hens laying 300 eggs a year are equally unnatural. Nature would not produce such animals because they could not obtain by their own efforts the large amounts of concentrated food needed for such production. The artificiality of our "improvement upon nature" does not affect the value of the meat, milk and eggs thus produced. Their food value is perfectly normal. The microbes which we might grow for food will not be more "unnatural" than our high bred dairy cows, pigs and hens.

The microbes have two great economical advantages over the animals: they grow more rapidly, and they require simpler food. A young pig or chicken may double its weight in a month, but a yeast cell will double its weight in 2 hours. To be sure, a yeast cell is much smaller than a pig, but we can start our yeast culture in a large tank with 500 pounds of seed yeast, and in 10 hours, we have 2,500 pounds. This can be repeated day after day, taking 500 pounds as seed yeast, and using the remaining 2,000 pounds as food. A yeast factory with ten fermenting tanks could furnish 10 tons of yeast per day. To provide the same amount of protein in form of pork, it would be necessary to kill 80 pigs a day, or 30,000 pigs in a year.

It is customary to speak of hogs, cattle and other meat animals as "converters of food." They convert plants which consist largely of starch and cellulose, with small amounts of protein and fat, into meat which consists largely of protein and fat, without starch or cellulose. Animals need protein, however, to make protein. We may say that they concentrate the plant proteins by digesting the starch, and eventually part of the cellulose, and changing some of it

into fat. Naturally, we must not expect that one pound of food protein gives one pound of meat protein. Much goes to waste, and a good share of the animal protein is in form of skin, intestine, bones and other non-edible parts of the body. At best, four pounds of feed protein give one pound of meat protein. But plant proteins are cheap, and meat protein is in great demand and high in price.

The yeast also needs food, and there is some waste in the growing of yeast, though no protein is wasted, but the yeast food is still cheaper than the food for animals. Yeast cannot digest starch, but we have different chemical or biochemical means of changing starch to sugar. In this respect of utilizing starch and sugar, yeast can be compared with pigs or chickens. But all animals need protein to make protein while yeast can make protein from ammonium salts, which are cheaper than protein. They *must* be cheaper because the farmer uses ammonium salts as a fertilizer to make the plants grow which change the ammonia into protein. The plants need a whole season to do this while the yeast can do it in a few hours. So, instead of having ammonia changed to plant protein which then changes in the animal to meat protein, with considerable loss, we can have the ammonia changed by the yeast into yeast protein, without any loss, in one-hundredth of the time. If we consider that yeast can live on starch like the animals, but can make protein from fertilizer directly and without loss, and in a much shorter time, it seems pretty certain that ultimately, yeast protein will become cheaper than meat protein.

In this way we could get all our concentrated protein finally without animals, but we could not get along without the plants. Yeast needs sugar or starch, and it seems highly improbable that we will ever be able to make these food substances cheaper than nature can do it.

The ruminants among our farm animals, the cows, sheep and goats, can digest cellulose too. Cellulose is very much

cheaper than starch, because it is the most common carbohydrate produced by all plants, and because it is indigestible for man as well as for most animals. Even bacteria are mostly unable to use cellulose, and the few that can use it grow very slowly. A rapidly-growing bacterium that could use cellulose as efficiently as the yeast uses sugar, and that would thrive on sawdust and ammonia would have a great chance of becoming domesticated and cultivated on a large scale. Even if the thus fermented sawdust would not be fit for human consumption, it might be a valuable concentrated food for pigs and dairy cows because of its high content of bacterial protein, and its very low price.

Chemical conversion of cellulose into sugar is possible. By heating finely powdered wood with dilute acids to very high temperatures under pressure, a good share of the cellulose is changed to glucose. It is pretty certain that during the war, Germany provided for some of its alcohol and of its food yeast by fermentation of wood sugar.

Molds are as good a source of protein as yeast and bacteria. The mycelium contains more materials of the cellulose type which is probably indigestible, but molds convert all the ammonia into mold protein. Since the molds are closely related to the mushrooms, they develop some flavors related to the delicate mushroom flavor. The manufacturers of Camembert cheese never fail to print on the label that the rind is edible. This rind consists largely of the mycelium of *Penicillium Camemberti*, and it has a very pleasant taste resembling mushrooms. Recently, feeding experiments have been made with the mycelium of *Aspergillus niger*. The young mycelium was dried and ground up to chicken feed, and it was then mixed with cracked corn, the idea being that the chickens would not know the difference and would eat the mold with the corn. But the chickens did know the difference, and ate the mold first, and only when that was gone did they start on the corn.

In the little table at the beginning of this chapter, it was

shown that meat had about the same protein content as the microbes, but even lean meat has much more fat. This is an important item. Next to protein, fat is our most expensive food, and the plants of the temperate zone where most people live produce relatively little fat. Our fat supply depends partly upon animal fats, and partly on plant fats imported from the tropics. No wonder that the bacteriologist has thought of extracting the fat from microbes.

Not all microbes produce fat, but certain bacteria, most yeasts and most molds store some fat in times of plenty, and some have been found to produce quite large quantities. By providing ideal conditions, certain mold species can store so much that 25 to 40% of the dry mycelium is fat. Yeasts will not store quite so much, but they produce considerable amounts of fat when aerated in a sugar solution twice as concentrated as that for growing bakers yeast. There is no doubt that fat can be produced from sugar or starch by microbes on a commercial scale, but it does not seem probable that it can be done cheaply.

Not very much energy and thought has been given to the problem of growing microbes for food. There is no doubt that it is possible. While it may be a long time before we change our food customs, it will not be very long at all before we grow yeasts, molds or bacteria in quantities to provide the concentrated feed for our livestock. If the necessity should arise through unforeseen world events, the people of the entire world could be provided with digestible proteins from microbes.

Chapter Eighteen

MAN AND ANIMALS AS PARASITES OF BACTERIA

The title of this chapter has not been confused by the printer, nor has the author been confused. It is an old story that certain bacteria can be parasites of man and animals, but the following pages will show the new story that man and animals are parasites of certain bacteria. The story is similar to the legumes as parasites of bacteria. The bacteria do not fare badly by playing host to animals. They are caught and held prisoner, but they are well fed and permitted to multiply, only certain substances of their cells are taken away from them by the animal which needs these parts for its own growth. These substances are mostly vitamins.

A newly-born child has no bacteria in its intestine, but as soon as the child takes milk, its intestine becomes filled with large numbers of a rod-shaped organism called *Lactobacillus bifidus*. Later, when the child takes other food besides milk, other bacteria become established in the intestine, and soon, *Bacterium coli* dominates. However, many other species are always present, streptococci, lactobacilli, bacteroides, veillonellas, and gas-forming clostridia. Their relative numbers fluctuate with the diet as well as with the health of the person.

Metchnikoff, the great Russian bacteriologist, became curious about the large number of Bulgarians who lived to a very old age, and concluded that this must be due to their large consumption of a special fermented milk. While working in the Pasteur Institute about 40 years ago, Metchnikoff found that the main organism of this sour milk was not a streptococcus, but a long rod which he called *Bacillus bulgaricus*. (It is now called *Lactobacillus bulgaricus*.) He believed to have good evidence that this bacterium produced sufficient acid in the intestine to suppress putrefaction, and to

create a favorable intestinal flora conducive to good health. Twenty years later, American bacteriologists began to doubt the efficiency of this organism. It was found that a close relative, *Lactobacillus acidophilus*, had a far better chance of surviving in the intestine, of becoming established and remaining there. So *L. bulgaricus* was bowed out and *L. acidophilus* was bowed in.

While Acidophilus Milk is not as fashionable today as it was twenty years ago, all these experiments with lactic acid bacteria leave no doubt that these bacteria are beneficial to our digestion. As long as the intestinal contents remain acid, there is no development of putrefactive types such as Clostridium whose activity in our intestine is not desirable.

The relationship between man and his intestinal lactic acid bacteria is too simple to be very dramatic. It does not even seem very important, and bacteriologists have wondered for many years whether the enormous numbers of other intestinal bacteria might perhaps play a more important role in our intestine. Experiments had been made long ago with aseptically hatched chickens and with guinea pigs aseptically born by caesarean operation. But the results were somewhat contradictory, and inconclusive. Also, the results obtained with newly-born animals may not apply to older individuals. The final answer could not be given until 1942, and to understand this, it will be necessary to discuss the relation between vitamins and microbes generally.

The first discovery of a vitamin was not made with animals or man, but with yeast. In 1901, Wildiers in Paris noticed that a pure culture beer yeast would not multiply in a solution containing minerals, ammonium salt and sugar, but when he added a very small amount of peptone, meat extract, beer wort or yeast extract, multiplication was normal. He proved that the compound which caused this good growth was not one of the common building stones of protein, not an amino acid, nor one of the mineral compounds. As he could not identify it chemically, he called

it *Bios*. This Bios had all the properties which we now attribute to a vitamin. It was an "accessory growth factor" as the modern physiological definition of a vitamin reads.

Wildiers' discovery caused a good deal of comment for and against Bios. The experimental facts were usually confirmed, but the explanations varied greatly. One believed that the poor growth in sugar solution without other organic matter was due to traces of copper in the distilled water; others thought that the yeast merely needed very much time to adapt itself to such poor food; still others found a toxin produced by the yeast which was more harmful in plain sugar solution. Only one investigator believed that Bios was really a chemical compound needed by the yeast, and that it was related to lecithin.

The Bios problem seemed to be an academic question of purely theoretical importance until the first vitamins were discovered in 1912. Then bacteriologists and biochemists realized that the Bios phenomenon was identical with that of vitamins, and the problem was attacked with new vigor from several different angles. The biochemical school of Toronto under Lash Miller's direction tried to determine the chemical nature of Bios and found it to consist of at least 7 different substances, of which inositol and thiamine were identified. R. J. Williams, now of Texas University, tried to use yeast to determine the amount of Vitamin B. Much time was lost in these years and much confusion was brought about because it was believed at that time that Vitamin B was a single chemical compound. The term vitamin B is now applied to about 10 different water-soluble vitamins. It was further believed that all yeasts, or at least all *Saccharomyces cerevisiae* have the same vitamin requirements, whereas we know now that they differ greatly. Some varieties of *Saccharomyces cerevisiae* grow well in sugar-mineral–ammonia solution without any vitamins or bios, but the variety F. B. cannot produce biotin, the variety O. P. cannot produce thiamin, the variety G. M. can make neither inositol nor

pyridoxine. Step by step, it was found how the different fractions could be separated, and when all fractions of vitamin B were defined chemically, new accessory growth factors were discovered. Williams found a new growth-promoting substance which he called pantothenic acid. Many biochemists doubted the existence of this compound for a long time, but now it is recognized as an important growth factor for mammals as well as for yeast, and its chemical structure is known. Kögl in Utrecht who had worked a good deal with the growth factors of green plants isolated a growth factor for yeast and bacteria which he called biotin. Later, another factor called "folic acid" was discovered.

Gradually, another phase became prominent in the vitamin studies. The realization that a good share of the human diet as well as of the animal feed was more or less deficient in some vitamins led to a search for materials especially rich in vitamins. Yeast was one of the substances containing a good amount of Vitamin B and D, and the chemical industry began to extract the vitamins from the waste yeast of the breweries. It was soon realized that many bacteria can produce vitamins in very large amounts, and for a while, it seemed as if the manufacture of vitamins would become a bacteriological industry. But it did not take the chemists very long to unravel the chemical structure of the vitamins, and to develop means of making them synthetically more cheaply than bacteria could, although dried beer yeast is probably still the cheapest source of some vitamins.

The vitamin production by some bacteria and not by others resulted in a new development, the analysis of vitamins by means of bacteria, which will be discussed in a later chapter. And finally came the revelation of the role of our intestinal bacteria in furnishing us with the needed vitamins.

As has been stated before, the great question whether our

intestinal bacteria are of any use to us could not be decided because it was impossible to kill these bacteria without killing the host of these bacteria as well. At last, the continued efforts of the chemical industry to find better sulfa drugs have resulted in two products which served this special purpose. They are quite toxic to bacteria, and probably to the cells of the human body too, but they are not resorbed by the intestine and pass unchanged through our alimentary canal, killing all or at least most of the bacteria on their way. These compounds are sulfa-guanidine and succinyl-sulfathiazole.

When young rats were fed a standardized, sufficient diet, they gained in weight normally, but when the sulfa drug was mixed with the food, they ceased to grow. For a while, the rats were not really sick, they showed no special symptoms, but began to lose weight, and finally they died. Other rats were fed the same diet and the same amount of sulfa drug, but liver extract was added which is rich in vitamins. These rats grew normally, and increased their weight despite the drug in their daily diet. Obviously, the sulfa drug was not toxic to the rats, but it had removed some necessary part from their diet which could be replaced by liver extract. Since the drug had killed the bacteria in the intestine, it was clear that the part that was missing in the diet was a product of the intestinal bacteria. Detailed experiments showed that the bacteria had furnished folic acid and biotin. When these are given to the rats, they grow quite normally although the sulfa drug is mixed in their regular food.

The simple conclusion of these very recent experiments is that we depend upon the bacteria in our intestine to supply us with certain vitamins which may not be present in our diet in amounts sufficient for our health. Therefore, if we disturb our intestinal bacteria, they may fail to provide the vitamins, and our health begins to fail.

Quite different from this very recently discovered depend-

ence of man on bacteria is the well-known role which bacteria play in the nutrition of the ruminants. Among the domesticated animals, ruminants are more numerous than any other group. Cattle, sheep and goats have been domesticated on every continent, and camels also belong to this family. The reason for this preference is probably that they are easy to feed. They can live on nothing but grass and hay. This simple diet is not sufficient for high milk production or for fattening, but those are not natural conditions, they are forced upon the animals by the hurried impatience and greed of man.

In nature too, the most numerous of the larger animals are ruminants, such as deer, buffalos, gazelles, antilopes, giraffes etc. They are probably so abundant in the world because they can digest cellulose which is indigestible to almost all other animals, and they can use cellulose only because certain groups of bacteria in their paunch pre-digest it for them. No other animal harbors these bacteria, for they work slowly and take their time, and they require certain conditions which other animals cannot offer. The ruminants contain these bacteria in their large preliminary digestive system of three compartments between the mouth and the real stomach. The food is usually swallowed without much chewing and passes into the first and largest compartment, the rumen or paunch. This is filled with a semi-liquid mass consisting of plant matter in various stages of digestion, and it contains large numbers of cellulose-fermenting bacteria. They become thoroughly mixed with the newly swallowed food, but cannot attack the cellulose very well until the animal, after some time, regurgitates the plant matter which has become clumped together, and "chews the cud." In this second, real chewing process, the plant cells are quite thoroughly ground to bits, and the bacteria get into all parts of the plant cells. In the ensuing fermentation process, the cellulose is changed to acids, mostly acetic, lactic and butyric acids which become neutralized in the slightly alkaline

liquid, and are finally resorbed by the animal and used for respiration or for growth. Thus the cellulose is first used by the bacteria, and the products of the bacteria are then used by the ruminant. The fermentation of cellulose takes a good deal of time, and with other animals, the food moves too quickly through the intestinal canal to allow of much fermentation and absorption of cellulose. With cattle, the food requires 3 to 10 days before the indigestible part of the food is excreted.

With the help of the cellulose bacteria, the ruminants not only can utilize cellulose, but they also can use the plant protein to greater advantage than other animals. Plant proteins are not as easily digestible as animal proteins, but the bacteria in the paunch can break them up into amino acids in order to build their own protein from them. They break up more than they need, and the ruminant can utilize what is left. Later, when these bacteria pass through the stomach of the ruminant, many of them are killed by the acid, and then they are digested and their protein is used by the cow like any other protein in the food.

As soon as the role of the bacteria in the rumen of the ruminants was recognized, experiments were begun to improve upon this role. The natural feed of the cattle, grass and plant leaves generally, contains only a relatively small amount of protein, and if the farmer expects rapid growth or plenty of milk, he must feed his cattle concentrates which contain much more protein, but which also cost correspondingly much more. Then the idea was conceived that perhaps the cellulose bacteria of the rumen might be able to make their own protein from cheap ammonium salts instead of expensive protein from concentrates. The experiment was tried, and it worked. Not all experiments were a full success, but gradually, feeding formulas were worked out which could be depended upon. The most important change was the substitution of urea for ammonia. It was found that the ammonium salts did not always agree

with the animals, but they thrived when urea was given instead.

Urea is as low in cost as ammonium salts, the cows like it better, and the cellulose-fermenting bacteria of the rumen can use it to build their own protein. As a result, hundreds of tons of urea have been used recently as part of the rations of milk cows, and agricultural experiment stations recommend the admixture of urea to cattle feed.

Without cellulose bacteria, the ruminants could not utilize cellulose, and would hardly find enough other food to support their large bodies. The bacteria could live without the animals, but the animals could not live without the bacteria. The ruminants must therefore be considered parasites of the bacteria, just as man can be considered a parasite of his intestinal bacteria.

The wood-boring insects also contain cellulose-digesting organisms. The most thoroughly studied case is that of the termites. They harbor a protozoon which digests the cellulose of the wood ingested by the termite, and the termite lives on the products of the protozoon's digestion.

Most of these investigations of the relationship between animals and their intestinal bacteria are quite new, and much more knowledge of interesting interactions between the largest and the smallest living beings is to be expected by the pursuance of these studies.

Chapter Nineteen

INDUSTRY HARNESSES BACTERIA

The chemical industries change raw materials which nature provides into substances which are wanted by man. They change coal to gas and tar, tar to dyes, and dyes to disinfectants. They change wood to cellulose, cellulose to nitrocellulose or cellophane or nylon, and so forth. Bacteria also change substances which nature provides to other substances. Molds change sugar to citric acid, yeasts change sugar to alcohol, and ammonia to protein, bacteria change sugar to lactic acid or propionic acid or butyl alcohol, and so forth. The chemical industries have found that bacteria can bring about certain changes of materials more easily than the chemist, and so they are employing bacteria for special purposes. The chapter on the Domestication of Yeasts has shown the vast extent of the manufacture of industrial alcohol for the chemical industry. Alcohol is one of the most common and indispensible solvents in chemical manufacture. Other materials used in chemical manufacturing, which can be made by bacteria, yeasts or molds, are glycerine, acetic acid, lactic acid, propionic acid, butyric acid, citric acid, butyl alcohol, and some other products of minor importance.

But it is not the chemical industry alone that depends upon the assistance of the microbes. The textile industry obtains some of its most valuable fibers with the help of bacteria, namely linen, hemp, and jute. The food industries already have taken up three chapters, so we will only mention here in passing that the fruits of the cocoa tree must be fermented before the beans can be made into cocoa or chocolate. Also, the berries of the coffee tree are fermented to set the beans free.

Bacteria, yeasts and molds produce enzymes which are

gradually finding commercial markets. Vitamins also are produced by them although in most cases, the chemical industry can manufacture them more cheaply.

Some of the industries which use bacteria are very large, some are very small, some are old, established industries while others are so new that they must be considered to be still in the experimental stage. All of them show in how many different ways our civilization, from linen handkerchiefs and a cup of cocoa to automobile lacquers and dynamite depends upon the cooperation of the much vilified microbes.

Microbes Making Textiles: Some textiles are manufactured by nature as separate single threads, ready to use, notably the cotton, the wool, and the silk. Other textiles are fibers imbedded in the stems or leaves of plants and must be freed from the plant material. With a few tropical plants, this can be accomplished by pounding and scraping alone, e.g. with the leaves of *Agave rigida* in Florida and Central America which furnish the so-called Sisal hemp. But with such plants as flax, hemp, and jute, the fibers are so glued together with other plant materials that the "glue" must be dissolved before the fiber can be freed. This is done by a process called "retting."

A hundred years ago, each farm family retted its own flax, and spun and wove its own linen. In Europe, many farmers still do that year after year. It is not a complicated process, it requires no knowledge of bacteriology or chemistry, it was a household craft which careful people with a keen sense of observation could bring to perfection while careless and dull people obtained an inferior product.

Two main methods have been used in the various countries, the water retting and the land retting or dew retting. The water retting is more common. It consists of putting the bundled flax or hemp completely under water in a pond or lake or a sluggish river. Rapid flow must be avoided. For the land retting, the bundles of flax or hemp are simply placed on the meadow or plowed land, and rain and dew keep

the plants moist. If they get too dry, they are sprinkled with water. By both processes, the fibers remain intact while the other plant matter rots away to a considerable extent, and the rest can be separated mechanically from the long fibers.

The two processes are quite different from the viewpoint of the bacteriologist and it seems surprizing that they can give the same result. The reason is the following: the fiber consists of cellulose, and the "glue" by which the fiber is fastened to the bark and pith of the plant is made of pectin. Pectin can be decomposed by many microorganisms while cellulose is attacked only by a few species. Among the bacteria which can destroy pectin are a number of anaerobic types, such as *Clostridium pectinovorum* and *Clostridium felsineum* and *Pectinobacter amylophilum*, but also many aerobic molds and bacteria.

When the plants are placed under water, some of the plant material will dissolve and furnish food for bacteria which multiply readily between the stems and leaves and soon have used up all oxygen in the water. This creates anaerobic conditions which are ideal for the development of *Clostridium* types. These bacteria are always present in small numbers in ponds and rivers, and they multiply rapidly in the mass of organic matter, digesting the pectin, and thus separating the fiber from the other plant matter. If retting is done continuously in the same place, it proceeds more rapidly because the right kind of bacteria accumulate, and the larger numbers of bacteria accomplish the decomposition more quickly.

It is important not to leave these plants too long in the water because cellulose-destroying bacteria also develop under these circumstances. The decomposition of cellulose is slower than that of pectin, and a correct timing of the retting process leaves the fiber quite intact. But it requires a certain experience and continuous watching to obtain a high grade material.

When the right stage is reached, normally after 2 to 3 weeks, the retted material is washed in running water. Most of the partly decayed plant matter sloughs off. The crude fibers are dried, and the remaining plant materials are removed by very primitive brakes and brushes on the farm and by elaborate machinery in the large textile industry.

The land retting offers quite different conditions for microorganisms. Molds can grow excellently under these conditions and the land retting seems to be largely accomplished by molds. Pectin is readily destroyed by many different molds, but most of them are also capable of attacking cellulose. The danger of weakened fibers is therefore greater in land retting. Another trouble may arise from molds which produce pigments, and thereby discolor the fiber so that it does not bleach readily.

When it was recognized that retting is due to microorganisms, attempts were made to obtain a better product with pure cultures. Sterilization of the plant matter by heat is too difficult, but the addition of certain pure cultures of pectin-fermenting bacteria has been quite successful. The Italian Carbone process uses *Clostridium felsineum*. A pure culture is grown in boiled potatoes, and is mixed into the water of a special retting vat. The water is kept at about 100°F., and the retting proceeds so fast under these conditions that it is usually completed in 2 days.

The water retting of hemp has been practiced for several thousand years from Northern China to Egypt. Jute, the fiber of a Bengal plant, is also obtained by this process.

Leather Manufacture: In the tanning industry, bacteria are used for certain initial steps of the complicated process. The hides are usually preserved for transport by pickling or dry salting. For tanning, the salt must be soaked out, and the hides cleaned from adhering flesh and blood by putrefying bacteria. The removal of hair from the hides is now usually done by quicklime, but formerly, the hides underwent a "sweating process" where bacteria at warm

temperatures multiplied rapidly on the skins and destroyed the roots of the hairs. Skins which are dehaired with lime are sometimes soaked in a bran infusion. The bran ferments by a mixture of bacteria and the result is lactic acid and other acids which neutralize the lime in the skins without danger of excess acid.

Tobacco Fermentation is a term frequently used in the curing of the green tobacco leaves to the brown product which is used for smoking or chewing. In the curing process, the dry leaves are stacked in piles and kept moist, and a change takes place which produces so much heat that the temperature rises to about 140°F. This change is largely due to enzymes in the leaves. Naturally, bacteria multiply too under such favorable conditions, and are present in large numbers. It is assumed by some bacteriologists that they influence the quality of the tobacco, but others can not agree with this, and consider the bacteria to be without influence.

Vinegar and Acetic Acid: The vinegar manufacture started like the alcohol manufacture originally as a food industry, but later developed into the source of an important raw material for the chemical industry. Acetic acid has very diversified uses in chemical manufacture.

Vinegar is made from alcohol by the vinegar bacteria which oxidize it to acetic acid. Vinegar for the household is usually made from some fruit juices which have undergone alcoholic fermentation, most commonly in this country from cider, although grapes, tomatoes or any other kind of fruit may be used as well as beer (so-called malt vinegar). As the process is an oxidation, much air is needed, and the rate of vinegar formation depends upon the rate of oxygen supply.

The old method of vinegar manufacture, sometimes called the French or the Orleans Process, is probably as old as the invention of wooden casks. .The cask, lying on its side with the bunghole open, is filled to about one-third with wine. The exchange of air through the one hole is very slow, and sometimes, a few smaller holes are bored into the head of

the barrel. On the surface of this wine, vinegar bacteria will multiply. Most common is a species whose cells secrete thick gelatinous membranes by which all bacteria stick together. They form a thick, coherent, slippery skum which is called mother-of-vinegar. Its bacteria oxidize the alcohol to acetic acid as rapidly as the air can come into the cask. This is so slow that after 3 to 6 months, only about one-third

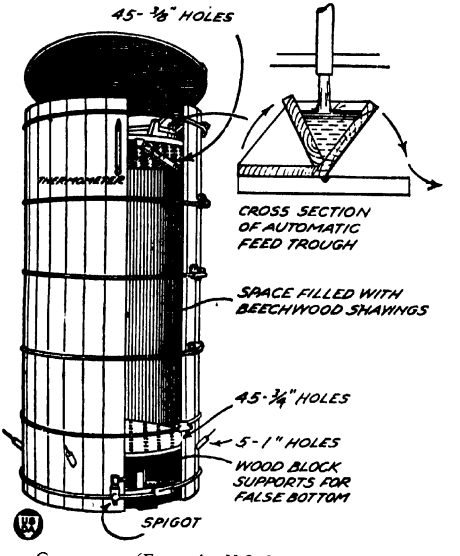


FIG. 95. Vinegar Generator. (From the U.S. Department of Agriculture Bulletin by E. LeFevre.)

of the vinegar can be drawn off, and a corresponding amount of new wine is poured through the bunghole so that the same bacteria can continue to make more vinegar.

Very different from this is the so-called Quick-vinegar or German process invented by Schützenbach in 1823. It provides a very large surface so that vinegar bacteria have an abundance of air. The generator consists of a round wooden tower, 5–6 feet in diameter and 15 feet high, with perforated bottom, filled with some loose, porous material over which the wine trickles down while air comes up through the holes

in the bottom. In the manufacture of cider vinegar, the material used for filling the generator is frequently corn cobs. The hard cider is uniformly distributed over the surface of the generator, and trickles continuously down over the corn cobs which are covered with a mass of vinegar bacteria. A good deal of heat is produced by the vinegar fermentation. This creates a draft, and the air rushes upwards, furnishing the bacteria with the much-needed oxygen. All vinegar generators are provided with thermometers, for the heat produced by this fermentation may raise the temperature so high that the bacteria die. When the thermometer indicates danger, the manufacturer cuts down the air supply by shutting off some of the holes.

Apples contain on the average about 10–12% sugar, which should give a hard cider of about 5–6% alcohol. Some of the alcohol and of the acetic acid is lost during the fermentation process, as anyone can confirm who ever visited a vinegar factory. The acetic acid in the atmosphere affects the eyes and lungs of the visitor very noticeably. Usually, cider vinegar contains between 4 and 5% of acetic acid.

As a rule, only about one-half to one-third of the alcohol can be oxidized by the bacteria in the short time in which the hard cider trickles from the top to the bottom of the generator. It would be impractical to make the generator two or three times higher to obtain a complete change of alcohol to acetic acid. Two different procedures are customary. Either, the partly oxidized cider is sent through a second and eventually a third generator to complete the oxidation. Or the hard cider is mixed at the very start with twice its volume of vinegar, and then one run will be sufficient to produce the finished vinegar.

Interesting is the manufacture of a much stronger vinegar containing as much as 12% acetic acid, which is made from distilled alcohol. Such strong vinegar is needed in the chemical industries. This process requires careful control of all phases. The generators are filled with beech shavings

specially prepared for this purpose. The alcohol is mixed with some vinegar at the start, and runs through the first generator, then through a second, and third and fourth, continuously ncreasing in strength. The bacteria in each generator become highly specialized, and if the vinegar from the first generator would go directly to the fourth, the bacteria would be completely upset by this unaccustomed food, and the generator would not function.

The bacteria which change alcohol to vinegar are somewhat different from the average type, and the bacteriologist places them in one genus called *Acetobacter*. There are at least a dozen different species, differing in form, in motility, in the type of membrane formed on the surface of the liquid, and also in the amount of acetic acid they can produce. The generators are sometimes started with pure cultures, and while the entire method of vinegar manufacture makes it impossible to exclude the entrance of other bacteria, the cultures in the generators are practically pure because no other bacteria could thrive in such an acid medium, with so little food aside from the acid and the alcohol.

Vinegar for table use is usually pasteurized to prevent any skum formation which might make the vinegar cloudy. The heating also kills the "vinegar eels," little nematodes barely visible to the naked eye which can live in vinegar by feeding on the vinegar bacteria. They are carried about on the feet of fruit flies which are greatly attracted by the smell of vinegar. Vinegar eels are harmless, but not appetizing.

Lactic Acid: A large number of bacteria can produce lactic acid from sugar. Naturally, for commercial manufacture, the type is chosen which will make the strongest acid, and that means one of the Lactobacilli. They can produce much more than the streptococci.

The raw material is sugar, and two cheap sources are available, the cane sugar of molasses and the milk sugar of whey. Whey is the liquid part of the milk that is left when hard cheese or cottage cheese or casein is made. It is of

little food value since most of the protein and all of the fat have been removed, but it still contains about 4% milk sugar.

The whey or diluted molasses is heavily inoculated with the Lactobacillus, and when it becomes strongly acid, lime is added which neutralizes the acid, so that the bacteria can produce more acid. This is continued until all sugar is changed to lactic acid. Really, the acid is mostly in form of the lime salt, calcium lactate. This salt crystallizes, and can be purified, and from the pure salt, the pure lactic acid can be obtained by a simple chemical process.

Lactic acid production is quite simple compared with other manufacturing processes. It is used in a number of food industries to acidify the food, in the dyeing of certain textiles, in leather manufacture and for the manufacture of plastics. The salts of lactic acid also have various uses.

Propionic Acid: This acid is produced by the fermentation of sugar through a group of bacteria called *Propionibacterium*. The first bacterium of this group was discovered some 40 years ago; it was the bacterium which made the holes in Swiss cheese. The milk sugar is fermented to propionic acid and carbon dioxide gas. The characteristic taste of Swiss cheese is also partly due to propionic acid.

The raw material for propionic acid manufacture is either molasses or whey. Fermentation must be carried on in pure culture.

There is some demand for this acid in the manufacture of solvents for lacquers, also in the perfume industry. Recently, it has been found that propionic acid can be used as an antiseptic in foods to prevent mold growth.

Citric Acid: Citric acid is the natural acid of all citrus fruits, and is made commercially from surplus lemons. But it is also made by certain molds, and the manufacture by molds is cheaper than the manufacture from lemons.

Certain strains of *Aspergillus niger* can ferment glucose or cane sugar to a mixture of acids of which citric acid is the main product if the living conditions of the mold are kept

just right. This fermentation is a partial oxidation. The mold needs air, and it is more difficult to grow molds commercially than bacteria, because molds do not thrive when submerged below the surface of the liquid. But industry has conquered these difficulties, and the manufacture of citric acid from molasses is a rather new but well established industry.

Citric acid is in considerable demand for pharmaceutical purposes, in the beverage industry, and also in the candy industry.

Gluconic Acid: This acid is also produced by molds through partial oxidation of sugar. The molds are different from the Aspergillus that makes citric acid, and different molds have different chemical mechanisms. The commercially used molds are *Aspergillus No.* 67, *Penicillium luteum* and *P. chrysogenum.*

Gluconic aci has certain pharmaceutical applications.

Gallic Acid is produced in a similar way by the oxidation of tannin by molds, usually *Aspergillus niger.* This acid is the starting material for certain dyes, and for ink. It has also some medicinal uses.

Penicillin, a product of *Penicillium notatum*, has been discussed in Chapter Eight.

Acetone and Butyl Alcohol: These two compounds are the main products of certain fermentations by sporeforming bacteria which are sometimes given the collective name "commercial solvents fermentations" because the Commercial Solvents Corporation is the leading company in their manufacture (see frontispiece).

Commercial production of acetone by fermentation was started during the first world war when the U.S. government needed large quantities of acetone for the manufacture of munitions. After the war, the butyl alcohol (or butanol) became prominent for the manufacture of quick-drying automobile lacquers. Acetone is still in demand as a solvent for the chemical industry.

FIG. 96. Top view of the fermenting vats at the Commercial Solvents Corporation plant, showing the pipes conducting fermentation gases to gas tanks

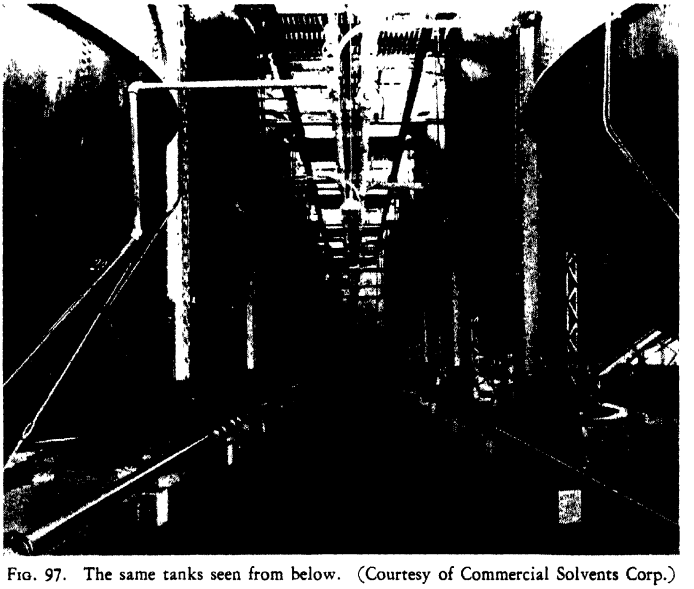


FIG. 97. The same tanks seen from below. (Courtesy of Commercial Solvents Corp.)

Two different types of fermentation are distinguished by the bacteriologist. One group of bacteria is aerobic although they can also live in the absence of air. *Bacillus macerans* had been found more than 50 years ago to ferment potato starch to acetone, ethyl alcohol and acetic and formic acids. The commercially used culture is *Bacillus aceto-ethylicus*. This bacillus makes no butyl alcohol, but makes acetone and the common ethyl alcohol. From 100 pounds of starch can be obtained 8–10 pounds of acetone and 20–24 pounds of alcohol. The bacillus ferments not only sugar, but starch and even corncobs if they have been heated under pressure with dilute acid, and 100 pounds of corncobs yield 2.7 pounds of acetone and 6.8 pounds of alcohol. Besides, large amounts of carbondioxide and of hydrogen gas are produced.

Butyl alcohol is produced only by strictly anaerobic bacteria of the Clostridium type which are killed by contact with the air. The commercial species is *Clostridium aceto-butylicum* or a closely related type. The Clostridium can ferment many different sugars as well as starch after it has been hydrolyzed. One of the customary raw materials is corn. From 100 pounds of corn can be obtained 16 pounds of butyl alcohol, 7 pounds of acetone and a small amount of ethyl alcohol. The fermentation must be carried out in absolute pure culture because even small contaminations may completely ruin the fermentation. The sterilization of starchy material in huge fermentation tanks of 50,000 gallons is no small task. The cultures must be "built up" gradually from a test tube culture through increasingly large volumes to small tanks of about 100 gallons capacity from which the large fermenting tanks are inoculated. All connecting pipes necessary for the transference of these 100 gallon batches to the big fermenters must also be absolutely sterile.

The fermentation is carried out slightly above 100°F., and lasts 2 to 3 days. The fermented mash is then distilled

by a very complicated process to separate the various fermentation products.

Acetone has very wide uses in the chemical industry. It is a solvent for fat, for cellulose acetate, for paints and varnishes, and it is the raw material for such materials as plastics, smokeless powder, artificial leather, and chloroform. The applications of butyl alcohol are equally diversified. It serves as a solvent for lacquers, dyes, oils, photographic films and is the raw material for synthetic resins, waterproofing compounds, and many other chemicals.

The gases produced in this fermentation are hydrogen and carbon dioxide. By passing them in a standardized mixture over a metal catalyst at high temperature, they combine chemically and form methyl alcohol, a very pure wood alcohol. This is another solvent greatly needed in the chemical industry.

Glycerine Fermentation: When soap is made from fat, glycerine is a by-product, and in normal times, enough glycerine is obtained in this way to supply the needs of the various industries. In times of war, however, the demand for glycerine is much higher because it is needed for nitroglycerine, the powerful explosive of dynamite. In times of war, the usual source of glycerine, namely fat, is likely to be more scarce. During the first world war, the great lack of fat compelled Germany to think of other materials for the much needed glycerine, and it was found that yeast, the regular alcohol-forming yeast, did ferment sugar into glycerine when the sugar solution was made alkaline. Ordinarily, yeast grows and ferments in an acid medium, such as fruit juice. In an alkaline medium, the yeast changes approximately half of the sugar into glycerine, the other half is fermented to alcohol, acetic acid and carbon dioxide gas. The fermentation permits glycerine manufacture on a large industrial scale, but in normal times, the price of glycerine as a waste product of soap manufacture is too low to pay for the expense of manufacture by fermentation.

Enzyme Preparations: It has been mentioned in several chapters that microorganisms can use insoluble materials such as starch, cellulose or fat only by secreting substances which decompose the insoluble materials to soluble products. These secreted substances are called enzymes. The best-known enzyme is probably pepsin which makes insoluble

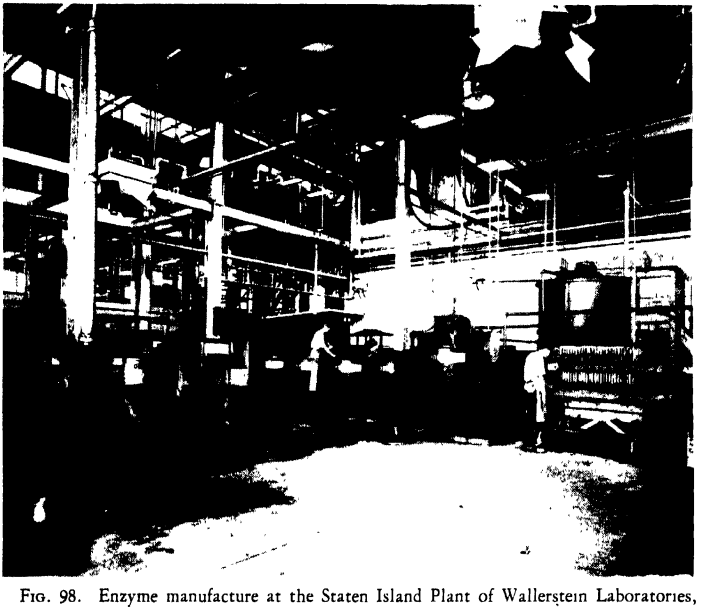


FIG. 98. Enzyme manufacture at the Staten Island Plant of Wallerstein Laboratories, New York.

proteins soluble. Then there is diastase (now usually called amylase) in our saliva which changes starch to sugar. All animals and all plants, including the microbes, use various kinds of enzymes in their metabolism.

Certain species of microorganisms produce some desirable enzymes in large amounts, and in such a form that they can be readily separated from the microbes and prepared in pure form. The starch-dissolving enzymes of molds can take the place of malt, pectin-dissolving enzymes of molds are used to clarify fruit juices, the protein-dissolving, pepsin-like

enzymes of *Bacillus subtilis* find various uses in chemical and food industries, also in the leather industry.

The manufacture seems simple in principle. It is only necessary to grow the bacteria in a suitable medium, then remove the bacteria and separate the enzyme from the liquid. Actually, very carefully controlled culturing is necessary, and the purification of the very sensitive enzymes is a very difficult task.

Chapter Twenty

INHERITANCE WITHOUT SEX

Each one of us assumes that there is not another person in the world just like himself, and he is probably right although there are two billion people in the world. Even twins are different to the close observer. To most of us, the cows in a large Holstein herd look all alike, but the people who care for them can tell them apart easily. The trees in a forest are all different, it is even impossible to find two absolutely identical leaves on the same tree. A robin can distinguish its mate from hundreds of others which show no differentiating details to our eyes. It seems quite justifiable to assume that each bacterium has its individuality, that it is different from all other cells in the same pure culture. We would not be able to differentiate between the individuals, but that is merely because we cannot see them completely enough.

Some differences among bacterial cells of the same species are conspicuous enough to be seen by careful observation. Every bacteriologist knows that there is a certain fluctuation in size and form among the individual cells of a pure culture. Some are longer than others, or a little thicker than others. Careful recording of all existing forms shows that the differences in form amounted to more than just differences in proportion. The opposite picture represents the various forms which Henrici found regularly in pure cultures of *Bacterium coli*. This bacterium is always described as a short rod, similar to No. I and III. However, all the other forms suggesting micrococci, or vibrios, or resembling the club-shaped and wedge-shaped cells of diphtheria bacteria are always present. They occur only in small numbers amounting to perhaps 1 to 5% of the total, but the picture is concrete evidence that the individual cells of a pure culture are by no means all alike.

However, these differences are not inheritable. They are fluctuations produced by chance differences in the environment. If a single vibrio form is separated from the others and grown by itself, its offspring are not different from that of a normal-shaped cell.

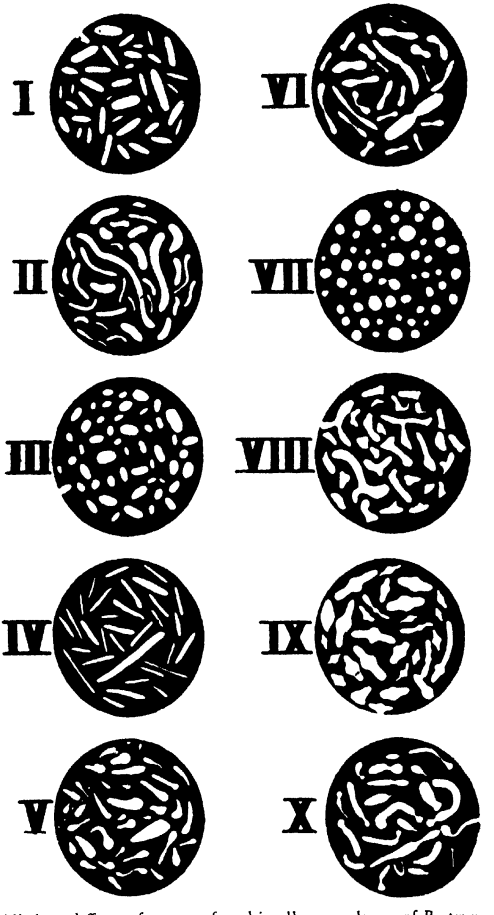


FIG. 99. All these different forms are found in all pure cultures of *Bactersum coli*. (From A. T. Henrici: Morphological Variation and the Rate of Growth of Bacteria 1928. Courtesy of Charles C. Thomas, Publisher, Springfield, Ill.)

Another individual difference can be studied, namely the time required by each single cell to double. By placing the bacteria on an agar surface where they cannot swim away, and by making a drawing or photograph of them every 5 minutes, we can determine when each cell divides, and when its offspring divide again. They do not all divide at the same time. While some individuals of a pure culture divided in 15 minutes after they were "born," others took 40 minutes, and occasionally even 70 minutes. All had the same food, light and temperature, but even twins showed great differences. However, these variations were not inheritable. When the offspring of the rapidly dividing cells were compared with those of the very slow ones, there was no difference. All variations were just fluctuations produced by chance.

This kind of variation is known to all of us. We cannot predict which puppy of a litter of high-bred hounds will be the best hunting dog, or which of the thousands of uniform wheat seeds we plant will bear the largest number of grains. We know their average under favorable conditions, and we know that there will be quite noticeable individual differences, however careful we are in preparing the seedbed and keeping all conditions uniform.

Besides these unpredictable chance variations, there are predictable differences, predictable because they are inheritable. The offspring of Golden Bantam corn are again Golden Bantam and not Country Gentleman or Howells Evergreen corn. These differences are constant, and as permanent as anything in biology is. Probably this type of corn did not exist 100 or 1000 years ago. It is the result of systematic breeding by man, a successful attempt to combine the good properties of one variety with the good properties of another. The question is how it happened that there were different varieties of corn to begin with.

Long observation and careful study has taught us that once in a great while, a plant or an animal gives rise to

offspring which is not only different, but which also passes this difference on to some or all of its offspring. The systematic study by geneticists of plants and animals has shown that such sudden changes occur with certain frequency. Some can be expected to happen in 1 out of 500 plants or animals while others happen only in 1 out of a million individuals. Such hereditary changes are called mutations. They are passed on to the offspring because they represent changes in that part of the organism which brings about multiplication. Mutations may happen in any cell. If they occur in a leaf cell of a tree or in a muscle cell of an animal, they will not be conspicuous, and will disappear forever when the individual dies. They are not passed on to the offspring. However, if they occur in the sex cells, the difference will be passed on to a new individual. All cells of this new individual originate from the one changed egg cell, and will have the changed character; therefore, all the offspring of this individual will display the new property, and thus a new variety is created.

Bacteria are not fundamentally different from plant or animal cells, and it seems only plausible to expect bacterial mutations. The difference between higher organisms and plants exists only in their mode of reproduction. In higher plants or animals, a mutation is passed on to progeny only if it occurs in the sex cells. With bacteria, it will be passed on if it occurs in any cell.

There is no doubt that mutations happen in bacteria; they have been observed frequently. Many more mutations happen which cannot be observed. If a rapidly growing bacterium produces mutants which grow slowly, the slow ones will be crowded out by the quick-growing variety, and though the new mutant may be present in our culture, we would never know it. Since we cannot distinguish between individual cells, as a rule, it will be only possible for us to detect such mutations which are favorable to the cell under the conditions of our culture methods.

An early well substantiated case was the observation by Neumann that once in a great while the orange colonies of *Staphylococcus aureus* produce one sector that grew white. From this, a white Staphylococcus could be obtained in pure culture which was identical with the orange form in every other respect. We might call it the albino form. Eventually, in long-continued cultivation, this white form did sometimes mutate back to the original orange variety. Colorless varieties of the red *Bacterium prodigiosum*, and of the green *Pseudomonas fluorescens* can be obtained by prolonged cultivation at high temperature.

The greatest attention has probably been given to the *Bacterium coli mutabile* which Neisser and Massini discovered in 1907. This resembled the typical intestinal *Bact. coli* in all respects but one; it could not ferment lactose. But when it was cultivated on lactose agar for a number of days, some of the old smooth colonies developed small, buttonlike secondary colonies, and this new growth consisted of cells which could ferment lactose. Once the cells had acquired this new property, they kept it for years. The original mutabile strain kept its property too: it did not attack lactose, but now and then produced a variant that could do it. According to an interesting investigation by I. M. Lewis, this happens in 1 out of every 450,000 cells. If only one corn plant out of 450,000 mutates, a good many acres must be planted before one such new individual appears. With 10 billion bacteria in an ordinary test tube, each tube would contain 20,000 mutated cells. In ordinary broth, these 20,000 are not different from the other 10 billion, and may become completely lost. But if we put lactose in the broth, the 20,000 variants have a better food than the others which cannot digest lactose; the new variety with the better food grows more rapidly and will finally dominate and crowd out the original strain.

This happens in nature continuously. Every ounce of soil offers different conditions of food, air, light, temperature

and moisture to the millions of bacteria which live there, and while one variety dominates in one spot, another variant may gain the upper hand a few inches away. In water, too, a good deal of difference exists between the upper and lower strata of a lake, or between the rapidly flowing and the stagnant parts of a river. As a result of this, the number of different varieties of bacteria is so great that it is quite confusing to the bacteriologist. It is often impossible to tell whether we have two different varieties of the same species, or two different species.

The formation of new varieties which may fit a new environment better than the original culture is usually called adaptation, but this term does not necessarily mean inheritable variation. Good use of adaptations is made in bacteriological industries. It is often possible to "train" a culture to produce more product than it did when freshly caught. Vinegar bacteria can "learn" to produce more acid, or yeast may be trained to produce more alcohol. Also, the type of fermentation can be changed, and a bacterium may be induced to make a different product. Microbes can be trained to live in conditions where they could not exist before, e.g. to grow at very high temperature or in a very concentrated solution. In the manufacture of alcohol from starch, yeast has been trained to tolerate a high concentration of sodium fluoride which is added to prevent the development of butyric acid bacteria. The concentration of sodium fluoride is so high that it would promptly kill an untrained yeast.

It has been known for a long time that bacteria may lose certain properties if grown with small amounts of antiseptics. *Bacterium coli* can be made to lose the ability to produce gas if grown with dilute carbolic acid. The new strain was tested for 2 years, and never regained the property of gas formation.

These older observations of mutations in microbes can now be explained by a direct chemical reaction of the antiseptic with certain proteins in the cell. The direct proof

has been obtained by the experiments of Steinberg and Thom. They found *Aspergillus* molds greatly changed when nitrite was added to the culture medium. First they lost step by step the various means of sexual and asexual spore formation, and sometimes it disappeared completely, resulting in a mycelium which merely grew hyphae. The explanation given is simple to the chemist. Nitrite is known to destroy amino groups. These groups are an important part of all proteins, and it is imaginable that in this way the molecules which control spore formation of the mold are incapacitated and cease to function.

Then these two investigators went one step further. They tried to restore the defunct amino groups back into the cell. It is difficult to decide what was more surprising, the boldness of such a thought, or the fact that it succeeded. By cultivating the mutated varieties with large doses of lysin which contains an excess of amino groups, they could reverse the mutations and restore, in some cases completely, the lost properties.

This accomplishment has opened up an entirely new method of approach to experimentation as well as to explanation of genetic changes. It is another example of bacteria taking the place of guinea pigs. Experiments of this type with higher plants or animals seem impossible, or at least they did seem impossible before the above experiments were carried out. Now that the trail has been blazed, thanks to the microbes, a relatively simple biochemical explanation of certain mutations has been found and proved experimentally, and sooner or later some method will be found to apply this new knowledge to higher plants or animals.

Bacteriologists are disturbed by another kind of variation which has been known for 60 years and has been studied intensively for nearly 20 years, but for which we still have no adequate or generally accepted explanation. It is usually called "microbic dissociation" and consists in a more or less sudden change of several properties simultaneously. To give

a specific example, the spore-forming anthrax bacillus, cause of spleenic fever, has long cells with square ends which form stiff threads on nutrient agar giving a characteristic wavy appearance to the colony. By cultivation at a temperature of about 105°, it changes sometimes (not always) into a bacterium with rounded ends which gives a soft, pulpy colony instead of the brittle dry form; it does not produce spores, and it has lost its virulence. Upon further cultivation, it may change still more, forming slimy capsules which

FIG. 100. Smooth and Rough Colonies of *Neisseria catarrhalis*. (From A. T. Henrici, Biology of Bacteria. Courtesy of D. C. Heath & Co., Boston.)

make the colony so watery that it flows when the agar is tilted. No bacteriologist would ever suspect it to be an anthrax colony, nor would he change his mind by looking at them through the microscope. They look decidedly different from the well-known *Bacillus anthracis*. These new types may occasionally revert back to the original type, or they may not.

The bacteriologist calls the first type which produces dry, flat colonies, the rough or R type; the soft, pulpy colonies characterize the smooth or S type, and the extreme, the transparent watery colonies are called the mucoid or M type. A fourth type, the gonidial or G type, has been claimed by several bacteriologists. It consists in extremely small cells, which pass the porcelain and diatomic earth filters that

hold back the normal-sized bacteria. They are hardly visible under the microscope, they grow slowly, their fermentation is different from that of the original culture, and they do not react equally with antisera.

For most groups of bacteria, the smooth type is the common status. Among the sporeformers, and also among the tubercle bacteria, the rough type is common. A few species are commonly in the mucoid type, e.g. the pneumococcus.

Bacteria change their properties by the shift from smooth to rough. They usually lose the ability to make pigments, and their fermenting properties are weakened. Bacteria which could produce gas from sugars may not do it in the R form. Pathogenic bacteria may lose their virulence partly or completely. All in all, they are so entirely changed that no bacteriologist would recognize them in that state.

Such changes may be brought about by old age, by too high temperatures, by inadequate nutrition, by too alkaline a medium, and by several other causes. They are sometimes noticeable by the formation of small secondary colonies on the full-grown original colonies, as mentioned above. So far, we have no definite rule by which to dissociate a bacterium. By trying one or the other method, it is frequently possible to dissociate a bacterium, but not always. More difficult yet is the reversion. Quite often, it has been impossible to reproduce the original culture from the dissociant. The confusion caused by this change of many properties is augmented by the intermediate stages between the S and R and M forms. The number of intermediates seems to be legion.

The importance of the knowledge of this dissociation is obvious when we realize that a dangerous pathogenic bacterium of the S type may change to a harmless R type and may thus live in the soil or water unsuspected, and yet, under certain conditions, could revert again to the pathogenic S type and cause an epidemic. In commercial fermentation where quality of the product is important, a dissociation can ruin the product.

Microbic dissociation has not as yet been satisfactorily explained. The comparison with the life cycles of insects or of malaria parasites is not very good because bacteria multiply readily at each stage of their "life cycle" while insects and plasmodia do not. In a recent article in *Science*, similar changes appeared to a bacteriologist "as if under appropriate environmental conditions, crows and robins should each mutate into blue jays, or pines and cedars metamorphose into redwoods."

This author makes the not unusual error of comparing bacteria with higher organisms, instead of with the individual cells of higher organisms. If we compare cell with cell, the higher organisms show a far greater variability than bacteria. A mammal originates from a single fertilized egg cell. This cell divides again and again. After while the new cells begin to show differences. By the time the animal is full-grown, we have muscle cells, blood cells, bone cells, nerve cells, liver cells, epidermis cells, leucocytes, and a number of other types, with enormous morphological and also physiological differences, greater than between any S and R and G forms of bacteria. And all these mammalian cell types are irreversible. A similar variety of different cell types is found in plants; root cells, leaf cells, epidermis cells, cells of the petals, the stamen, etc., all derived from one single cell, and most of them quite as irreversible as those of the mammals. The term "microbic differentiation" instead of dissociation might have found much less opposition and more understanding among biologists.

The analogy with the cells of higher organisms shows that "dissociation" is nothing new or abnormal. But we do not understand it as yet. In higher organisms, differentiation is due to hormones, but no hormones are known to exist in bacterial cultures. A clear explanation of microbic dissociation would be of great help to the industries which employ bacteria, and it may give us a better understanding of the origin, the progress and the vanishing of epidemics.

Chapter Twenty-one

MICROBES REPLACE GUINEA PIGS

Instead of debating the similarity and the differences between microbes and guinea pigs, let us start right away with an example. It will be easy to show that many thousand guinea pigs, rats, pigeons and other animals formerly needed for vitamin assays are saved every year because bacteria take their places.

Vitamin Analysis: We have seen in an earlier chapter that the first vitamins were not discovered with man or animals, but with yeast. A certain beer yeast would not grow in sugar solution with ammonia and minerals, but if a very small amount of orange juice or vegetable juice or meat extract was added, it grew well. The amount of these extracts was so small that it could not have served as food in the common sense of the word. We usually call these substances vitamins. The correct biochemical term is "accessory growth factors."

The measurement of the amount of vitamins in food was very difficult 30 years ago when the chemical nature of the vitamins was not known. To determine, for example, the amount of vitamin C in carrots, guinea pigs had to be fed several weeks with a diet free from vitamin C until they showed plain symptoms of scurvey. Then, the animals were fed carrots, each animal getting a different amount. From the rate of recovery, the amount of vitamin C in the carrots could be estimated in arbitrary units. The same procedure was necessary for other vitamins. Pigeons were used for vitamin B, rats for vitamins A and D. To make a single determination, a number of test animals had to be fed without vitamin for a considerable time, until they showed symptoms of avitaminosis, and from the amount of the new

food that cured the symptoms, the vitamin content was recorded in "units" which were not very definite.

Since that time, the chemists have found out the structural formula of practically all vitamins, and the amount of vitamin is not given any more in "standard units," but by weight. Also, the knowledge of the chemical composition has made it possible to determine some of them by chemical analysis. Vitamin C is always determined chemically, and vitamin B_2 (riboflavin) can be measured optically because it fluoresces. With all other vitamins, chemical analysis is not only very slow, but also inaccurate because the very small amounts of vitamin in food substances are mixed with so many different compounds that separation is not always complete.

It was a clever idea to substitute molds, yeasts, and bacteria for the guinea pigs, pigeons, chickens, monkeys etc. All vitamins are growth factors. Microbes need vitamins for their growth just as much as the higher organisms. They may not need all of them. No bacterium, yeast or mold is known to need the fat-soluble vitamins A and D. Strangely, none of them needs vitamin C either. But the many different B vitamins seem to be very important for all microbes. This does not mean that they cannot grow when the vitamins are absent. They have such great constructive powers that some of them can make all necessary vitamins and do not need any in their food. *Bacterium coli* belongs to these, and we have already seen that we live on the vitamins produced by this bacterium in our intestine. Many soil and water bacteria also can produce all vitamins needed, and most molds can do it too. But other groups of bacteria, like the *Lactobacilli* and the *Streptococci* have not the skill to make all vitamins. Among the yeasts, some can make everything, some can make all vitamins but B_1, others make all but B_2, and so forth. This difference in vitamin requirement is used for "vitamin assay," i.e. for measuring the amount of any vitamin in a material.

In order to determine the amount of a vitamin in any substance three things are needed; (1) a pure culture which needs this vitamin; (2) a small quantity of the pure vitamin; (3) a culture medium which contains everything that this pure culture needs for growth except the vitamin. In a testtube with this medium, the pure culture will not grow at all. If we put a little vitamin in, we have little growth, and with more vitamin, more growth.

If we wish to know the amount of thiamin (B_1) in tomato juice, we use *Lactobacillus helveticus*. In our special medium, the bacterium does not grow, but with 0.1 micrograms of thiamin, there is a little growth, with 0.2 micrograms the culture is definitely cloudy, and with 0.5 micrograms, the maximum turbidity is almost reached. The turbidity is measured accurately by the amount of light that can pass through the culture onto a photo-electric cell. In this way, a certain turbidity indicates a certain amount of vitamin. Then to a parallel set of tubes with the vitamin-free medium is added varying amounts of tomato juice instead of vitamin. In these tubes, the amount of bacterial growth will depend exclusively upon the amount of vitamin B_1 in the tomato juice. If one tube with tomato juice is just as cloudy as our tube with 0.2 micrograms of B_1, we know that the tomato juice in that tube contained 0.2 micrograms thiamin.

Such an analysis is done in 36 hours, as compared with several weeks by the old animal method, and the amount of labor and material cost involved is very much less. The results are more accurate than animal tests, and are given in micrograms rather than arbitrary units.

The following vitamins can be analyzed by microbiological methods: Thiamin (B_1), Riboflavin (B_2), Nicotinic Acid Amid, Pyridoxin, Panthothenic Acid, Folic Acid, Inositol, and Biotin. The turbidity measurement cannot be used if the substance under test makes the medium cloudy as for instance milk would do. Then, the fermentation products of the organism are determined, either the amount of acid

in the case of lactobacilli (these organisms are used for several vitamins) or by the volume of carbon dioxide when the test organism is a yeast. With the mold, the entire surface growth is removed, washed, dried, and weighed.

Analysis of Amino Acids: In Chapter Four, it has been shown that all proteins are built up from amino acids. For the study of normal growth, and also of abnormal growth as in the development of tumors and cancers, it is often necessary to know the amount of different amino acids present. But their separation and measurement is quite difficult and inaccurate because they are chemically so much alike. Nevertheless these slight, inconspicuous differences are the cause of all the diversity between the proteins of different organisms.

Bacteria contain proteins like all other organisms, and they must build them up from amino acids. The green plants can manufacture all amino acids themselves, and need none from other sources. They make them from the carbon dioxide of the air and the nitrate and water of the soil, by some unknown process, with the help of sunlight. Animals, quite to the contrary, can make only a few amino acids, and must have most of them in their food ready-made in order to grow. If only one is missing, they will not grow.

Bacteria stand between these two extremes. Some need no amino acids; they can use ammonium salts or nitrate and sugar, and build their protoplasm, containing many different amino acids, entirely from these simple compounds. The common *Bacterium coli* belongs to these efficient bacteria. Others may need just one amino acid, but can make all others. The dysentery bacteria can produce all amino acids except tryptophane, but do not grow without this complex compound. Other species must have a number of amino acids ready-made. *Lactobacillus arabinosus* cannot make glutamic acid, tryptophane, threonine, valine, leucine, isoleucine, cystine, lysine and phenyl alanine. That might give the impression that this lactobacillus is most difficult

to cultivate. But we must remember that not only these 9 amino acids, but many others also are present in most of the common proteins, and are regular constituents of such substances as meat extract, peptone, casein, blood serum and other materials which we ordinarily use to make bacteria grow.

The analysis of amino acids is modeled after the vitamin analysis. For instance, a medium is made which contains everything that *Lactobacillus arabinosus* needs for growth except glutamic acid. A number of tubes of this medium is prepared with increasing amounts of glutamic acid; the *Lactobacillus* will grow better with more glutamic acid. Then the unknown substance is added to some other tubes of this medium, and the intensity of growth of the Lactobacillus is proportional to the amount of glutamic acid. Equal amount of growth means an equal amount of glutamic acid.

This method of analysis is just in the beginning stage, but it has been worked out for some acids, and will probably be applicable for all amino acids.

Sugar Analysis: Microbes are also used in sugar analysis, and though this does not save guinea pigs, it shows the wide usefulness of microbes. The methods are mentioned here because they are closely related to the preceding analytical methods where microbes are taking the place of chemical reagents.

The oldest case is the determination of sugar in the urine of diabetic patients. The doctor puts the urine into a fermentation tube called saccharimeter, and puts a little piece of bakers yeast in it. If any sugar is present, the yeast will ferment it, and from the amount of gas that collects in the tube, the doctor can tell how much sugar the urine contains. Though all modern hospitals have laboratories where the sugar can be analyzed by chemical methods, this simple fermentation test is still in use since it can be applied any-

where without any laboratory equipment. Our figure is taken from a very recent catalog of a medical supply house.

One of the great difficulties of sugar analysis is the separation of the different sugars. Plants usually contain several different sugars, and it is important for certain purposes to determine the amount of each separately. The common cane sugar, called sucrose by the chemist, is easily split into two different sugars, glucose and fructose. Maltose,

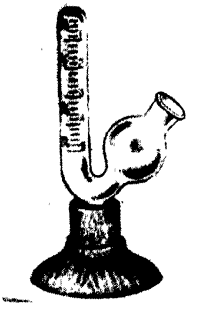


FIG. 101. The Eichorn saccharimeter to measure the sugar in urine.

the malt sugar, can be split into two equal parts of glucose. Lactose, the milk sugar, breaks up into glucose and galactose. These are the most common sugars, but a good many others exist. They are all so nearly alike that they give the same reactions with most chemicals, and if we wish to determine how much raffinose, sucrose and glucose is found in the juice of the sugar beet, that is a difficult problem for the chemist.

The bacteriologist can come to the assistance of the chemist by furnishing him with cultures of bacteria or yeasts that decompose only one of these sugars. Some varieties of yeast and some lactic acid bacteria will ferment all glucose, but do not change the other two sugars. The chemist determines the total sugar at the start, lets the bacteria ferment the glucose, and the difference between the original amount and what is left must be glucose. Then he adds

another variety of yeast or bacteria which ferment sucrose, but not raffinose. Now, since glucose and sucrose have been destroyed, the remaining sugar must be raffinose.

This may sound a little easier than it is in actual practice, but this is a method now used for certain sugar analyses. The pure cultures which will select and destroy one certain sugar among a number of others are known and can be bought commercially.

Microbes for the Study of Human Physiology: It is obvious that we cannot use bacteria to study heart troubles, kidney stones or brain tumors, or the normal or abnormal function of any organ as a whole, because bacteria have no organs. For these cases, guinea pigs will have to continue their role of saving human lives. But the body of all animals consists of cells, and whenever functions of the individual cells are concerned, it may be easier to study the problem with the cells of yeasts or bacteria than with animal cells. The fundamental functions of most cells are very much alike, and there is so much variation in the physiology of bacteria that it is usually possible to find one possessing just that very property that is to be studied.

In several respects, for such studies bacteria are preferable to guineapigs. A culture of bacteria consists of only one kind of cells whereas a guineapig has epidermis cells, nerve cells, muscle cells, liver cells and so forth, all of them different chemically and physiologically. A single organ or tissue dissected from the guineapig may contain only one kind of cells, but the cells will die in a few hours after separation from the body.

It is possible to use tissue cultures for certain experiments. A very small portion of tissue dissected from a living animal can be made to grow if it is kept aseptically in a solution containing blood serum or a similar body fluid. Only very small pieces of tissue can be cultivated in this way, rarely more than a quarter inch in diameter. These tissues grow slowly. They need more than a day to double in size while

bacteria do this in one hour. The experimental difficulties of growing tissue cultures prevent their general use for physiological investigations, and bacteria and yeasts are the chief organisms on which some of the modern studies of cell physiology have been made.

Forty years ago, when the law of conservation of energy was applied to human and animal nutrition, and calories became popular, the law was tried out also on bacteria and yeasts. The cultures were placed in a calorimeter, the food consumption was measured, the heat production registered, and as was to be expected, the law of conservation of energy was found to apply to bacteria as it did to all other organisms. Perhaps not much knowledge was gained by these experiments, but bacteriologists and biochemists became aware of the similarity between a bacterial culture and an animal. Experimental possibilities were realized; a trail had been blazed.

The chemical changes taking place in the muscle during work and rest had always been an intriguing problem to the physiologist, but difficult of approach. It was known that lactic acid was formed in this process which later disappeared. Then it was suddenly realized that some bacteria also form lactic acid, and the study of the change of sugar to lactic acid was much simpler with bacteria than with muscle cells. It was found that lactic acid in the muscle was produced by a special protein, called an enzyme, very similar to that found in Lactobacillus. Then it was discovered that in the alcoholic fermentation by yeast, the sugar is at first decomposed in the same way as in the muscle, but when a certain stage is reached, the yeast has some enzymes differing from those in the muscle or in the bacteria. Enzymes are very special proteins which bring about gradual chemical changes. In the course of nearly 30 years of research by several of the leading human physiologists who worked with a large staff of associates, these two related processes, the lactic acid formation from sugar and the

alcohol formation from sugar, have been almost completely unraveled. What was supposed 30 years ago to be one enzyme, is now known to be a sequence of some seven different enzymes, and each enzyme needs one or two co-factors, i.e. other compounds which are not active in themselves, but without which the enzymes cannot act. Among these co-factors are phosphate and magnesium salts, and some other co-factors were finally proved to be vitamins. Thus it became known why vitamins were necessary, and how the extremely small amounts of vitamin in our food can play such an important role in our body.

At present, when the alcoholic fermentation and the functioning of the muscle can be considered quite well understood, physiologists have concentrated their attention on respiration. Here again, microbes are the main experimental organisms because all different stages of complete and incomplete oxidation can be found in the various bacteria, yeasts and molds, and again, the mechanisms of respiration in the highest animals and in the simplest bacteria are found to be very similar.

As we progress further and further in our studies of physiology and biochemistry, we find more and more similarities between the life mechanisms of the highest and the lowest organisms. This refers also to plants which differ from animals above all by their chlorophyl with which they can change light into growth energy. But the plants too have enzymes similar to those of the yeasts and the animal muscles, and their respiration is also comparable with that of animal cells or bacteria. Nature apparently uses the same approved mechanisms in most organisms, and creates the inexhaustible variation of the different animals and plants which populate our earth, simply by small additions or omissions in the intricate mechanism of the single cells.

Chapter Twenty-two

THE WORLD WITHOUT MICROBES

An Epilogue

The interrelations between microbes and the higher plants and animals, including man and his civilization, have been shown to be so manifold and diversified that some readers might want a short summary. Instead of repeating briefly and monotonously the contents of chapter after chapter, let us realize the good and bad features of microbes by visualizing a world without them. Let us imagine what would happen if suddenly each and every microbe on earth were killed, perhaps by a collision of the earth with the tail of a comet which contains a mysterious gas killing all microbes without doing any damage to plants, animals and man.

Our first reaction is a sigh of relief for we think first of the freedom from contagious diseases. People sick from typhoid, diphtheria or meningitis or other microbial diseases will recover quickly except where vitality is too low already to overcome the damage done by toxins or other bacterial poisons. We rejoice over the certainty that no new cases can develop, no epidemics will ever threaten humanity again. We can change the tuberculosis hospitals to old people's homes, and the leprosy colonies to public bathing beaches. Quarantine and vaccination exist only in old fairytale books, and sound like medieval torture to the new generation. The farmer will be happy that his livestock is safe now from glanders, contagious abortion, hog cholera and chicken diarrhoea. And how his crops will thrive now, without danger from blight or wilt or leafspot or tumors! It sounds like a bumper crop each year.

And our food will keep now. We can discard the refrig-

erator, for now, meat will not become smelly, vegetables will not rot, oranges and apples will not mold, cider will not ferment. If we only protect our food from drying and from too much light and air, food will keep. No more need for canning, drying, pickling, freezing! It sounds almost too good. What an easy life.

Of course, there are some drawbacks. If food cannot spoil, cabbage cannot change to sauerkraut. We might make it by putting vinegar in the cabbage, but there is no vinegar because all vinegar bacteria were killed by the comet. Well, we can make vinegar chemically, for instance by dry distillation of wood. It has not the flavor of fruit vinegar, it is chemically pure acetic acid diluted with water, but it is at least sour, and we could add artificial flavors.

Speaking of vinegar reminds us of the hard cider from which vinegar was formerly made. All yeasts are dead; there is no hard cider, no beer or wine. But there is still industrial alcohol. That can be made chemically; some alcohol is now manufactured in this way. Baking powder can be substituted for the bread yeast.

We can buy a whole month's supply of milk and cream because it keeps. But as it does not sour, we have no buttermilk, and no cottage cheese. If lactic acid can be made cheaply by the chemist, it could be added to the milk to make cottage cheese. With vinegar, it would not taste so good. And as to hard cheeses like cheddar which are now ripened by bacteria, we might learn to produce a similar result by adding pepsin.

As long as our cows give plenty of milk, we need not worry about dairy products. They may taste a little different, but the food value would be the same. But will the cows continue to give plenty of milk? Cows can digest cellulose, and live largely on grass and hay because some bacteria in their complex stomach predigest the cellulose for them. Now that these bacteria are dead, cellulose is indigestible to cattle, and they must be fed like pigs and

chickens with a more concentrated food, such as grains and seeds and oil cake, perhaps even meat scraps. That is expensive feeding.

The farmer will have to raise more corn and alfalfa and beans than ever before, to make up for the loss of cellulose digestion by the cattle, and at first glance, the prospects of large crops look very good because there are no more plant diseases. But let us take a second glance, at the soil where the roots of the plants take up the minerals and nitrate and send it to the leaves to be transformed into proteins. There is only very little nitrate in the soil at any one time. Most of the soil nitrogen is in reserve, in form of humus which is decomposed but very sparingly by bacteria, and furnishes a slowly, but continuously flowing source of nitrate. Without bacteria, this source cannot flow. The nitrogen is locked up, and the key is lost. After the plants have used up the small amount of nitrate which was in the soil when the earth collided with the comet's tail, there is no more nitrate, nor any other nitrogenous plant food. The nitrogen-fixing bacteria are also dead, and the legumes must have nitrate like all other plants.

To prevent the plants from starving, we must fertilize the fields frequently. Then we make a very disappointing discovery: the barnyard manure does not fertilize. It contains no nitrate, not even ammonia which some plants could use. It does not smell as it did in the days before the comet. It contains many valuable substances which bacteria could very quickly change into plant food. But as there are no bacteria, the plants can use none of the substances in the barnyard manure.

The only thing that will keep the plants alive now is nitrate. Fortunately we have learned to make nitrate from the nitrogen and oxygen of the air by means of electricity, and it is manufactured in enormous quantities. Nitrate will be the most important industry in post-comet days because all life on earth will depend upon our ability to keep the

plants alive. Without nitrate no plants, and without plants no food for man or animals.[1]

Nitrate production may become enormous, but it is limited by the available electricity, and our ability to distribute the nitrate also has its limits. It will not be possible to bring it to every corner of the world. The large forests in northern Michigan and in the Rocky Mountains cannot be provided with nitrates, and it is unavoidable that they will die within a few months. In fact, very large areas of our country will become deserts, not from lack of water, but of nitrate; and the green areas will be oases surrounding the more densely populated parts of the country while the rest becomes a desert. In countries whose industry is not as far developed as ours, the population will be starved to death in a short time. The same fate would have happened to us if the collision had occurred fifty years earlier, because nitrate manufacture is a very recent industry.

We can now provide enough nitrate to produce crops for our food, and we might continue a fairly normal life for a while, with our normal supply of coal and water power, with iron and gasoline, with automobiles and airplanes. We will live to see the Rocky Mountains studded with dead trees which will sooner or later be destroyed by unavoidable forest fires. We may not live long enough to see all the fertile soil, which is no longer held in place by grass and tree roots, being washed into the creeks, so that only bare rock remains where Yellowstone Park used to be.

We may not live long enough to see this happen because a peculiar, almost unknown disease will befall all of us within a few months after the collision. It is an avitaminosis. The bacteria in our intestine which provided us with certain vitamins, are dead. Our regular food does not contain quite enough for our needs, and our store becomes

[1] It is worthy of note that now, the plants depend entirely on man to provide them with plant food. Heretofore, plants were independent, and man dependent on plants. Now we have true symbiosis where one cannot live without the other.

gradually exhausted, and we become ill. If sufficient amounts of the needed vitamins can be made by chemical factories, we can continue to live. If not, there is still a chance of extracting enough of the vitamins from the dwindling plant growth on earth. If that fails too, human life on earth ceases entirely, and with it, the last traces of plant life would also disappear. All life on earth has come to an end because the microbes died.

But if we can replace the vitamins formerly furnished by the intestinal bacteria, we can maintain human life, and national life, and a civilized life. We certainly cannot afford to go back to stone age civilization. Only a highly industrialized people can survive under this predicament of no microbes. So many things will become so entirely different that the new, microbe-less civilization must appear very artificial indeed to the survivors of pre-comet days.

One more great change has not been mentioned yet, and that is the difficulty of water supply. There is as much water on the earth after the comet collision as there was before, but it will be a different water. At present, all water in all rivers and lakes is continuously contaminated with sewage, and that cannot be changed after the bacteria are dead, because of the enormous volumes of water involved. All sewage must ultimately go into rivers, lakes or oceans. As long as we had bacteria, they decomposed the organic matter of sewage slowly, but completely. Some towns dumped all their sewage, after preliminary purification, into a river, and other towns, farther down the river, used that same water for their water supply without fear or loathing because it had become completely purified, thanks to the bacteria. Now that bacteria have completely disappeared from the face of the earth, there is no danger from contagious intestinal diseases, no danger of bad odors from putrefaction, no danger to aquatic life through exhaustion of the oxygen in the water. But all that just indicates that the sewage, after flowing down the river 50 or 100 miles, is still sewage, that

means a murky liquid made cloudy by finely suspended particles of human excreta, and containing relatively large amounts of urea. The sewage emptied into the Mississippi at St. Paul arrives weeks later at New Orleans in the same condition, only more concentrated by the sewage from all communities along the river, including Chicago. It is unavoidable that in a short time, all lakes and even the oceans will contain sewage in very noticeable amounts. This sewage does not smell, and people might to some extent overcome the feeling of repulsion for fecal matter, but it does not seem very probable that swimming in sewage, even in diluted sewage, would be a very popular sport.

As to the drinking water, the solution is simple. Rain water is as pure as ever, and all house owners could construct their roofs so as to catch the largest amount possible, and store it in cisterns. Deep wells may become as precious as oil wells. Our water supply will be smaller, but it will be good.

That is the prospect of life without microbes. It will seem a strange life to us who take the cooperation of microbes for granted, and it will be a hard life, but probably we can make it. Although we will be safe from any contagious diseases for ever, life without microbes may seem hardly worth living to most of us.

Let us hope that we never collide with the tail of such a comet.

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